Dear colleagues and friends,

On behalf of the Local Organizing Committee, we want to warmly welcome you to Maastricht for the 43rd International Symposium on Halogenated Persistent Organic Pollutants – Dioxin2023. This marks the first return of the meeting to Europe in the last 5 years and comes 12 years after the memorable Dioxin 2011 edition in Brussels! We are proud to continue the tradition of the symposium series as an interdisciplinary forum for communicating scientific advances and emerging issues, all in a friendly atmosphere. We are especially delighted as we are reviving the true essence of the event by holding an international face-to-face meeting in Europe.

As expected, Dioxin2023 will focus on the science of persistent organic pollutants (POPs) from a broad perspective. This includes not only the well-known legacy dioxins, PCBs, PBDEs, but also the more emerging flame retardants, PFAS, CPs, PCNs, and related compounds.

The symposium will cover a wide range of topics, such as analytical advances, emission/formation control, waste/risk management, environmental levels, fate and transport, human exposure, toxicity, toxicokinetic, food/feed, regulation, microplastics, and forensics. Additionally, we will explore the increasing attention given to developing countries and global pollution issues.

We are thrilled to announce that to date, we have received over 400 abstract/short papers from 40 different countries, out of which 225 have been scheduled for oral presentations in up to 5 concurrent sessions, and 175 posters that will be on display throughout the entire week. All congress materials are available for download on our website. This diverse programme will span over 4 days of conference, starting with a plenary lecture on a general topic of concern and concluding by a poster session.

The symposium venue, the MECC, is conveniently located just a few minutes walk from the city center and all major hotels. Moreover, your congress badge grants you free access to public transportation in Maastricht. As part of the symposium’s tradition, several social events have been scheduled throughout the week.

These events provide numerous opportunities to foster interactions between students, academics, and science professionals from all around the world. We encourage you not to miss out on these networking opportunities.

Once again, we extend our heartfelt gratitude to the more than 25 sponsors and exhibitors who have supported the symposium, making Dioxin2023 possible. Many of them will have booths in the exhibition area. We urge all participants to take the chance to visit their booths and learn about the latest developments in the products and services they offer. The exhibition will be an integral part of the symposium, with all coffee breaks, lunches, and poster sessions taking place in this area.

This year, both the scientific and social programme will culminate on Thursday night at the closing gala dinner. Thanks to the support of Wellington Laboratories, we have arranged a memorable evening in a unique venue where all delegates are invited to enjoy dinner and participate in the following party. This will provide us all with even more opportunities to connect with our colleagues and students.

Once again, on behalf of the local Organizing Committee, we extend a warm welcome to Maastricht and to Dioxin2023. We wish you all an exciting and enriching week!

On behalf of the Local Organising Committee

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To Mike and Mehran, our late friends, mentors, and colleagues.

'Ve believe in an afterlife. Simply because energy cannot die, it flows, transforms and never stops.' – Albert Einstein
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All abstracts listed in Dioxin2023 Symposium Book of Abstracts have been assigned a prefix for the type of presentation, and a sequential abstract number.

Oral Communication = Prefix based on the day, time slot, session and order or the presentation
E.g. MON-AM-A1 (Monday morning, session A, first presentation)

Poster = P
E.g. P-001

In the Book of Abstracts’ section, you will first find the Oral Communications, listed by day, topic and time of presentation. You will then find the all the Posters, ordered by topic (alphabetically).

Index of Presenters
The Index of Presenters lists all abstract presenting authors in alphabetical order. To locate the page of the abstract, first take note of the abstract number and then locate the abstract, listed by type of presentation and in sequential order.

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**MON-AM-A1 Occurrence of PFAS in the Belgian Food Chain**

Virginie Van Leeuw, Guillaume Fosseprez, Adrien Murphy, Laure Joly*
Sciensano, Rue Juliette Wytsman 14, 1050 Brussels, Belgium

**Introduction:** Human exposure to Per- and polyfluoroalkyl substances (PFAS) can occur through multiple pathways, of which food has been identified as a dominant source. In 2020, the risks related to PFAS were reassessed by the European Food Safety Authority (EFSA)\(^1\). Consequently, a TWI for the sum of PFOS, PFOA, PFHxS and PFNA, further called “4 regulated PFAS”, was established at 4.4 ng/kg body weight (bw)/week. Since a higher exposure of 12 ng/kg bw/week for the sum of PFOA and PFOS was determined for the Belgian population in 2007-2010\(^2\), the exposure to PFAS in Belgium should urgently be re-evaluated. This presentation will provide information on the occurrence of PFAS in the Belgian food chain and their concentrations.

**Materials and Methods:** The comprehensive sample collection resulted in 280 samples, reflecting all foods relevant for PFAS exposure and Belgian consumption habits. In addition, special attention was given to the selection of game meat, offal and egg-containing products. Different food matrices were selected for optimization and validation of the analytical methods, depending on their physico-chemical properties: (i) animal-origin tissues including fish, seafood (FIS), meat and meat products (MEA) (except organs), (ii) plant-based products including fruits, vegetables and cereals, (iii) dairy products, (iv) livers, (vi) eggs, and (vii) water. A QuEChERS-based extraction was developed for groups i-iv, while an acetone-based extraction was applied for v-vi groups, and no extraction was applied for water (vii). A two-step purification using solid-phase extraction (SPE) was optimized on Bond Elut PFAS WAX cartridges in combination with Bond Elut Carbon S cartridges. Analysis was performed by liquid chromatography-high resolution mass spectrometry in combination with an Orbitrap Q Exactive (LC-HRMS, Thermo) to analyze 24 negatively charged PFAS, including most of the advised European substances: 4 regulated PFAS, other carboxylate-PFAS (C5-C14), sulfonate-PFAS (C4-C13), and PFAS substitutes (DONA, F53B minor and major forms and HFPO-DA).

**Results:** The method validation was carried out according to the EURL Guidance document on analytical parameters for the determination of per- and polyfluoroalkyl substances (PFAS) in food and feed\(^3\). The applicability of the optimized protocols was assessed for each of the described food groups via repeated analyses (n=3) of samples fortified at minimally three concentration levels. Within-laboratory reproducibility was respectively £ 20 % and 25% for the four regulated PFAS and the other substances, respectively. Most of the limits of quantification, defined as the lowest validated level, ranged from 0.005 to 0.05 µg kg\(^{-1}\), depending on the compounds and matrices. Next, the validated method was applied to the selected samples, demonstrating widespread PFAS contamination in various foodstuffs. At least one PFAS was quantified in 73 %, 33 % and 29 % of the FIS, MEA and baby food (YNG) samples, respectively. No PFAS were detected in the nine egg samples independently of their origin (caged, free-range, organic). An average of 1.3 compounds per sample (ranging from 0 to 9), with concentrations ranging from <LOQ to 2.85 µg kg\(^{-1}\), was found among the 100 FIS, MEA, YNG samples. The average relative contribution of the four regulated PFAS to the sum of the 24 analyzed PFAS was 74 %, ranging from 0 % to 100 % among the 100 samples of FIS, MEA, YNG. PFOS was the most detected compound. Only sulfonic or carboxylic molecules with short carbon chains (≤ 8 carbons) were quantified in water samples.

**Discussion and Conclusion:** Although PFAS are omnipresent in food, all quantified results were below the maximum levels set by the Commission Regulation (EU) 2023/915. Next, the results can be used for a dietary exposure assessment for the Belgian population to PFAS.

**Acknowledgments:** The financial support was provided by the Federal Public Service (FPS) Health, Food Chain Safety and Environment, project RT 121/6350 – FLUOREX.

**References:**
MON-AM-A2  Analysis of Per- and Polyfluorinated Compounds at Low ppt-level in Fruits and Vegetables

Ruben Kause*, Stefan van Leeuwen, Ron Hoogenboom, Bob van Dooren, Rens Keppels, Helgah Makarem Akhlaghi, Leontien de Pagter-de Witte, and Bjorn Berendsen

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Introduction: Per- and polyfluorinated compounds (PFASs) are a group of substances that are widely used in various applications due to their exceptional chemical properties. However, their extensive use has led to their presence in the environment and food products. As PFASs can be harmful even at low concentrations, it is important to detect and quantify them accurately. In this study, a method was developed to detect and quantify 19 PFASs down to the low and sub-ppt-level (pg/g) in different categories of fruits and vegetables (leaf crops, fruits, tuber crops, garlic/onions, and ‘other’ vegetables), which were validated using matrix fortified calibration lines. Low pg/g-level detection limits were obtained by (1) increasing the sample intake, (2) lowering the solvent volume in the final extracts, and (3) implementing a very sensitive ultra-performance liquid chromatography – triple quadrupole mass spectrometric (LC-MS/MS) system. The developed method was applied in a large-scale study of vegetables from allotments in the vicinity of two large PFAS facilities in the Netherlands. The study was performed on over 800 samples of various local fruits and vegetables.

Materials and Methods: 10 g of fresh sample was weighed, and internal standards were added. The extraction was carried out using methanol. After shaking, ultrasonication and centrifugation, the extract was combined with water. The samples were then cleaned and concentrated using solid-phase extraction (SPE; weak anion exchange). The final extract was fortified with injection standards followed by detection by LC-MS/MS. Separation was performed on a mixed-mode C18 column with positive surface modification with methanol, and an ammonium acetate buffer (20 mM) as the mobile phase. The method was validated on 19 PFASs, including perfluorinated carboxylates (PFCAs), -sulfonates (PFSAs), GenX, and a few other PFASs. The validation was performed on six fruits/vegetables in each of the five categories and tested for LOQ, recovery, precision, and linearity. The quantification was based on matrix fortified calibration lines prepared in fruits or vegetables of the same category. Solvent blanks were used to correct for self-induced contaminations, and an isolator column (C18) placed after the LC mixing chamber was used to delay any contaminations introduced during injection. All materials used in this study were pre-tested for PFAS contamination.

Results: The method proved to be quantitative for the PFCAs, with chain lengths C5 – C14 and C16 (excluding C13, due to the lack of an isotopically labeled internal standard), and the PFSAs with chain length C4 – C10, as well as for GenX, NaDONA, 9Cl-PF3ONS, and 11Cl-PF3OUDs. The method can reliably quantify concentrations over three orders of magnitude, with the lowest quantification limits being 0.5 pg/g (0.5 ppt). Blank contributions were found mostly for PFOA and PFPeA, at the approximate levels of 1 – 10 pg/g and 10 – 100 pg/g respectfully, although some variation was noticed. The method showed very good performance criteria overall, with an apparent recovery of 90 – 119 %, a precision of 3 – 28 % and LOQs ranging from 0.5 – 100 pg/g.

Our method was used in a large-scale study on homegrown fruits and vegetables near two PFAS facilities in the Netherlands. The study found that fruits and vegetables near the two sites had higher levels of GenX and PFOA, which may be linked to emissions from the facilities. Samples from allotments in close proximity to one of the sites (<1 km) exhibited significantly increased levels of PFOA and GenX, with concentrations reaching up to 5 ng/g. In general, leaf crops, tuber crops, and fruits vegetables had higher concentrations of PFOA and GenX compared to other produce. Additionally, tomatoes and peppers showed higher concentrations of PFPeA near one of the facilities. As regards other PFASs, we detected elevated concentrations of PFHxA, PFHpA, PFUnDa, PFBS (25-500 pg/g) and some lower concentrations of other PFASs (0.5-25 pg/g). Produce from allotments further away from the sites (>1 km) showed significantly lower concentrations of PFASs.

Discussion and Conclusion: The developed method accurately detects and quantifies PFASs in fruits and vegetables at low and sometimes sub-ppt-levels. This study emphasizes the importance of monitoring PFAS contamination in food products, particularly in areas near contaminated sites like PFAS facilities. While the study found similar concentrations as previous studies on produce near contaminated sites [1,2], we were able to detect PFASs at much lower concentrations, which is essential for establishing current and possible upcoming stringent regulations. These results help to establish the population’s exposure to PFASs from consuming vegetables grown near industrial areas. Furthermore, the improved sensitivity of the method will aid in evaluating the contribution of fruits and vegetables to consumers’ overall PFAS intake.

Acknowledgments: The authors acknowledge the local authorities close to the PFAS facilities for their support and Arcadis for management of the project and providing the samples for the study.

References:
Per- and Polyfluorinated Substances (PFAS): Occurrence and Exposure
S. Van Leeuwen & Y. Yao

MON-AM-A3 PFAS and first-time EU maximum levels in food: (non)practical applicability for the food business by an analytical service provider

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Introduction: Per- and polyfluorinated alkyl substances (PFAS) have been in focus of the European Food Safety Authority (EFSA) for several years amongst others 1 and 2. Following the publication of a tolerable weekly intake of 4.4 ng/kg body weight and week (group-TWI) for perfluorooctane sulphonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA) and perfluorohexane sulphonic acid (PFHxS) – in the following called EU4-PFAS – maximum levels for selected PFASs in certain foods have come into force for the first time in the European Union (EU), taking effect from 1 January 2023 3. These maximum levels are related to eggs, fish meat, crustaceans, and bivalve molluscs as well as to meat and edible offal (related to fresh weight).

Analytical service providers perform chemical analysis of PFAS along the whole food supply chain (including primary production, processing, distribution, and retail). In this context, not only raw materials but also processed intermediate products and composed final products are part of the interest of farmers, food producers, retailers and / or authorities and research institutes.

Looking on food samples, analysed for PFAS by such an analytical service provider in 2018 until March 2023, the aim of this study is to check if these new maximum levels are applicable for the food business sector.

Materials and Methods:

Sampling: A dedicated sampling campaign was not performed for this study. But food, feed and biota samples were received from food and feed business operators, authorities, research institutes for analysis of PFAS. For this study, data collected from 2018 to end of March 2023 was taken. More than 5600 samples were identified as being for food purposes and allocated to different matrix categories on basis of the available data. For each matrix category applicability of EU maximum and indicative levels was defined.

PFAS-analysis in food: Samples were analysed by Eurofins GfA Lab Service, Hamburg, using an analytical method being part of the accreditation of the laboratory according to DIN EN ISO/IEC 17025:2018. In brief, samples were spiked with a mixture of 25 isotope-labelled-quantification standards (including $^{13}$C$_4$-PFOS, $^{18}$C$_8$-PFOA, $^{13}$C$_5$-PFNA and $^{18}$O$_2$-PFHxS) before extraction. Extraction was carried out by means of ultrasound using an appropriate mixture of polar organic solvents, followed by a multi-stage clean-up of the extract (QuEChERS / column chromatography (including activated carbon, ion exchanger)). Measurement was performed by liquid chromatography/tandem mass spectrometry (LC-MS/MS, Agilent Technologies 6495C LC/TQ coupled to 1290 Infinity II LC). Quantification of analytes using internal and external standards (multipoint calibration). To minimize the load of blank values, materials such as fluoropolymer plastics and glass materials were avoided in sample handling (storage, extraction, cleanup, etc.). The same applied to the measuring instruments (seals, inlet filters, degassers) by using stainless steel and polyetheretherketone (PEEK) materials. Quality control / assurance was performed by continuous control of laboratory blank values, regular check of certified reference materials / in-house reference materials and steadily participation in international laboratory proficiency testing schemes.

The used analytical method fulfills the requirements set by Regulation (EU) 2022/1428.

Results:

Overview on current EU-legislation for PFAS in food: Maximum levels for PFAS in food were set by the EU in December 2022, entering into force on 1st January 2023, and are related to the following PFAS-substances: PFOS, PFOA, PFNA, PFHxS and Sum of EU4-PFAS.

Maximum values are related to raw products of the primary production phase in the supply chain. For food which is dried, diluted, processed or compound food (i.e. composed of more than one ingredient), the following aspects shall be taken into account when applying these maximum levels to such food:

1. changes of the concentration of the contaminant caused by drying or dilution processes;
2. changes of the concentration of the contaminant caused by processing;
3. the relative proportions of the ingredients in the product;
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According to the procedure of Article 2 of Commission Regulation (EC) No 1881/2006 ⁶ resp. subsequent to this regulation Article 3 of Commission Regulation (EU) 2023/915 ⁷, these factors shall be provided and justified by the food business operators.

In addition to these maximum values so called indicative levels are set for further investigation of the causes of the contamination when such levels are exceeded ⁵ (see table 1 for details on the food categories). In terms of applicability, it is assumed here, that concentration or dilution factors resp. concentration factors will be considered in the same way as for maximum levels.

A complete overview on the current EU legislation concerning PFAS is given elsewhere ⁸.

Table 1: Matrix categories of EU maximum levels and EU indicative levels for PFAS in food (as appearing in this study)

<table>
<thead>
<tr>
<th>Foodstuff category</th>
<th>Direct applicability</th>
<th>Concentration / dilution factor needed</th>
<th>Processing factor needed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eggs</td>
<td>Fresh eggs (shell eggs and liquid whole egg)</td>
<td>Dried egg products (whole egg powder, egg yolk powder, albumen powder)</td>
<td>Fresh egg products (egg yolk and albumen)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Salted egg</td>
<td>Cooked egg products</td>
</tr>
<tr>
<td>Maximum level</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fish meat</td>
<td>(Fresh) muscle meat of fish, but information on fish species and purpose of use (food for infants and young children yes or no) needed</td>
<td>Dried muscle meat of fish (information on fish species and purpose of use needed in addition)</td>
<td>Canned fish products (additional ingredients)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fish (dried/salted/cured/smoked)</td>
</tr>
<tr>
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<tr>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Crustaceans and</td>
<td>(Fresh) meat of crustaceans and bivalve molluscs</td>
<td>Dried meat of crustaceans and bivalve molluscs</td>
<td>Not occurring in this study</td>
</tr>
<tr>
<td>bivalve molluscs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
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<td></td>
</tr>
<tr>
<td>Meat and edible</td>
<td>Raw meat and offal, but information on animal species needed</td>
<td>Dried meat</td>
<td>Cooked and / or breaded meat, meat products (e.g. sausages)</td>
</tr>
<tr>
<td>offal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indicative level</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fruits, vegetables,</td>
<td>Raw fruits, vegetables, starchy roots and tubers, funghi</td>
<td>Concentrated or dried fruits, vegetables, starchy roots and tubers, funghi</td>
<td>Extracts, canned products, processed products (protein, flour or starch)</td>
</tr>
<tr>
<td>starchy roots and</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tubers, funghi</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk</td>
<td>Raw, whole or skimmed milk</td>
<td>Dried milk powder</td>
<td>Milk products like cream, yoghurt, cheese, whey etc.</td>
</tr>
<tr>
<td>Baby food</td>
<td>Ready-to-eat-meals, biscuits, puffed products</td>
<td>Not occurring in this study</td>
<td>Not occurring in this study</td>
</tr>
<tr>
<td>(except fish meat)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 1: Increasing interest / need for PFAS-analysis in food in comparison with publication of relevant documents of EFSA ¹ ² and EU-Commission ³ ⁵
Per- and Polyfluorinated Substances (PFAS): Occurrence and Exposure
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Increasing interest / need for PFAS-analysis in food: Numbers of received samples being identified as food samples in this study are shown in figure 1, summarised in quarters per year from 2018 to 2023 (2023: only quarter 1). 47% (37%) of the samples were received in the last nine (six) months of this study showing the highly increased interest resp. need of the food business in analysis of PFAS in food. This comes along – by chance or not cannot be determined here in more detail – with the publication of the EU-Recommendation on monitoring of PFAS in food by the EU member states 5 and of Commission Regulation (EU) 2022/2388 of 7 December 2022 setting maximum levels of perfluoroalkyl substances in certain foodstuffs 3.

Applicability of PFAS-maximum and indicative levels in daily practice of an analytical service provider: Applicability of PFAS-maximum and indicative levels was defined for each identified matrix category in this study and results are summarized in tables 1 and 2 as well as in figure 2. EU-maximum levels for PFAS are directly applicable on 19% of the samples in this study, for EU-indicative levels 21% of the samples are directly.

Discussion and Conclusion:
The diversity of matrices, counted as food samples received by an analytical service provider, shows a growing interest of the food supply chain in PFAS levels in food beyond the existing EU-maximum and indicative levels. The limited number of food categories, for which legislative levels already exist in the EU, as well as the absence of concentration or dilution factors resp. processing factors significantly restrict the applicability of these legislative levels.

Acknowledgments: We thank the laboratory staff of Eurofins GfA Lab Service in Hamburg for their dedicated work during all the years.

References:
8. Eurofins Food News: Per- and polyfluorinated alkyl substances (PFAS): Maximum levels for PFAS in food came into force for the first time on 1 January 2023 (https://www.eurofins.de/food-analysis/food-news/food-testing-news/maximum-levels-for-pfas-in-food/ (March 2022))
### Table 2: Applicability of EU maximum levels and indicative levels on food samples in this study

<table>
<thead>
<tr>
<th>Food stuff (categories)</th>
<th>Total number of samples</th>
<th>Applicability of maximum level</th>
<th>Applicability of indicative level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>directly-applicable</td>
<td>directly-applicable but information needed</td>
</tr>
<tr>
<td>Egg &amp; egg product</td>
<td>303</td>
<td>68%</td>
<td>19%</td>
</tr>
<tr>
<td>- Egg product, fresh</td>
<td>235</td>
<td>88%</td>
<td>2%</td>
</tr>
<tr>
<td>- Egg product, dried</td>
<td>57</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>- Egg product, cooked</td>
<td>11</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>Fish / shellfish / other marine organisms</td>
<td>595</td>
<td>45%</td>
<td>40%</td>
</tr>
<tr>
<td>- Fish</td>
<td>406</td>
<td>38%</td>
<td>58%</td>
</tr>
<tr>
<td>- Fish product</td>
<td>53</td>
<td>91%</td>
<td>9%</td>
</tr>
<tr>
<td>- Crustacean</td>
<td>61</td>
<td>95%</td>
<td>5%</td>
</tr>
<tr>
<td>- Shellfish</td>
<td>52</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>- Shellfish (dried)</td>
<td>5</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>- Other marine organisms</td>
<td>18</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>Product of animal origin</td>
<td>392</td>
<td>66%</td>
<td>7%</td>
</tr>
<tr>
<td>- Meat</td>
<td>223</td>
<td>84%</td>
<td>6%</td>
</tr>
<tr>
<td>- Meat product</td>
<td>64</td>
<td>3%</td>
<td>3%</td>
</tr>
<tr>
<td>- Offals</td>
<td>81</td>
<td>84%</td>
<td>16%</td>
</tr>
<tr>
<td>- Other products of animal origin</td>
<td>24</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>Product of plant origin</td>
<td>1141</td>
<td>100%</td>
<td>49%</td>
</tr>
<tr>
<td>- Fruits</td>
<td>192</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>- Fruits (processed)</td>
<td>13</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>- Vegetables</td>
<td>363</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>- Vegetables (dried)</td>
<td>36</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>- Vegetables (processed)</td>
<td>18</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>- Mushrooms</td>
<td>19</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>- Mushrooms (processed)</td>
<td>2</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>- Grains</td>
<td>103</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>- Grains (processed)</td>
<td>208</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>- Shell fruit / nut</td>
<td>68</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>- Shell fruit / nut (processed)</td>
<td>8</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>- Oilseed/protein supplier</td>
<td>32</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>- Herbs</td>
<td>22</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>- Spices</td>
<td>6</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>- Other products of plant origin</td>
<td>51</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>Milk &amp; milk products</td>
<td>1345</td>
<td>100%</td>
<td>35%</td>
</tr>
<tr>
<td>- Milk</td>
<td>471</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>- Milk product</td>
<td>874</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>Food for particular nutritional uses</td>
<td>360</td>
<td>7%</td>
<td></td>
</tr>
<tr>
<td>- Baby food</td>
<td>360</td>
<td>7%</td>
<td></td>
</tr>
<tr>
<td>Oils &amp; fats</td>
<td>415</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>- Vegetable Oil</td>
<td>120</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>- Animal fat (incl. marine oils)</td>
<td>289</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>- Other oil / fat products</td>
<td>6</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>Tea, coffee, cocoa and herbal tea</td>
<td>37</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>Beverages and alcohol</td>
<td>135</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>Processed &amp; composite food</td>
<td>539</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>Additive / ingredient</td>
<td>344</td>
<td>100%</td>
<td></td>
</tr>
</tbody>
</table>
Per- and Polyfluorinated Substances (PFAS): Occurrence and Exposure

S. Van Leeuwen & Y. Yao

MON-AM-A4  Levels and spatial profile of perfluoroalkyl substances in edible parts of shrimp products from coastal areas of Japan and neighboring countries

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Introduction: Perfluoroalkyl substances (PFASs), such as perfluorosulfonic acids (PFSAs), PFSA with 8 carbons is perfluorooctane sulfonate (PFOS), and perfluorinated carboxylic acids (PFCAs), PFCA with 8 carbons is perfluorooctanoic acid (PFOA), are a concern for human exposure due to their long retention and some adverse effects. PFSAs and PFCAs have been detected in invertebrates, fish, and marine mammals worldwide. Several reports have indicated a decrease in PFOS levels over time, in contrast to PFOA concentrations that have tended to increase in tissues of aquatic organisms at many locations. Our previous reports (Fujii et al., 2019; 2020) indicated that long-chain PFCAs (9 to 14 carbons (C9 to C14)) were widely distributed in fish and shellfish, such as Pacific cod and clam, from Japanese coastal waters and that such seafoods are a major dietary source of PFCA exposure for humans. However, no survey on PFSAs in seafood has been conducted. The aim of the present study was to investigate the levels and profiles of both PFSAs and PFCAs in edible crustacean products (shrimp and krill) for human consumption.

Materials and Methods: Dried shrimp products were collected from 8 different areas along the coasts of Japan and other Asian countries (Taiwan, China, and Vietnam). The small pieces of shrimp products included Sakura shrimp, Akiami paste shrimp, Alaskan pink shrimp, and krill. The target chemicals were five types of PFSAs, including sulfonamides, and nine types of PFCAs (C6-C15). Dried shrimp samples were powdered and homogenized with methanol and tetrabutylammonium hydrogen sulfate/carbonate buffer (pH 10), and then extracted with methyl tert-butyl ether. The residue was derivatized for PFCAs using benzyl bromide. The derivatized PFCAs and underivatized PFSAs were separated by a silica gel column, eluting with hexane for PFCAs, followed by acetone for PFSAs. PFCAs were analyzed using GC/MS, while PFSAs were analyzed using LC/MS/MS.

Results: All five PFSAs, including sulfonamides, and nine PFCAs were detected in 30 dried shrimp products from the Japanese market. The mean concentration of PFSAs in the products was 6.5 ng/g dry weight, ranging from <LOQ to 28 ng/g dry weight. The mean concentration of PFCAs was 16 ng/g dry weight, ranging from 4.1 to 41 ng/g dry weight. Based on the total concentration in all shrimp products, the percentage contribution was accounted for as 28% for PFSAs and 72% for PFCAs. The major analogous were higher in the order of PFUnDA (25%) > PFTrDA (18%) > PFOS (18%) > PFNA (8.6%) > PFHpS (6.4%). Regional and species differences were observed in the levels and profiles of PFASs. Alaskan pink shrimp (Pandalus eous) from the Hokuriku coastal area was highly contaminated with long-chain PFCAs. In sakura shrimp (Sergia lucens), the concentrations of both PFSAs and PFCAs were about twice as high in Japan (Suruga Bay) as they were in the Taiwan coastal area. PFAS levels in krill (Euphausiacea Pacific) from the Sanriku coast were lower than those in any other species. There was no significant correlation between PFSAs and PFCAs, indicating a different emission source for both PFASs.

Discussion and Conclusion: Although the investigation of PFOS survey in seafood has been limited thus far, this study reveals that PFASs are still present in marine life, and the levels of long-chain PFCAs are higher in crustaceans (shrimp and krill) compared to fish (Pacific cod) and shellfish (clam) in Japanese coastal waters. The composition of PFSAs and PFCAs in shrimp is similar to that in the human diet and serum (Kärrman et al., 2009), suggesting that seafood, such as shrimp, may be a significant source of dietary exposure in Japan. Considering the broad distribution and food contamination of PFASs and the relatively high contribution of PFOS and long-chain PFCAs in the seafood market, their source identification needs to be further investigated for food safety.

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2. Fujii, Y et al., Environmental pollution 2020;263:114369
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Introduction: The production of homegrown food, including homegrown eggs through the housing of free-ranging laying hens for egg production, has gained increased popularity1. However, homegrown eggs in private backyards have been associated with elevated PFAS concentrations, both in industrial and rural residential areas even without any known nearby PFAS source2. The soil is a major sink of PFAS and a suggested major (in)direct exposure source to many terrestrial organisms, including free-ranging laying hens3. Experimental research has recently demonstrated that PFAS sorption onto the soil organic and mineral fractions is largely influenced by diverse soil characteristics and complex physicochemical interactions3. However, very little is known about its implications on the bioavailability of PFAS to terrestrial organisms under real-world field exposure conditions. Therefore, there is an urgent need for empirical models under real-world field conditions that can provide knowledge for proper risk assessment of PFAS and derived remediation techniques4.

The main objective of this study was to gain mechanistic insights into the potential role of soil concentrations and main soil physicochemical characteristics in explaining bioavailability of PFAS to homegrown eggs. Furthermore, empirical models were developed to predict PFAS concentrations in homegrown eggs, based on these variables. Lastly, the contribution of potentially relevant exposure sources (soil, rain water, earthworms and locally grown vegetables) to the egg PFAS concentrations was assessed.

Materials and Methods: To this end, the aforementioned matrices were sampled in 91 private gardens across Flanders in 2019, 2021 and 2022. These matrices were analyzed for 29 target PFAS and the following soil characteristics were measured in the soil from the chicken enclosures: organic matter content (OM), clay content, pH, exchangeable cations (i.e. K+, Na+, Ca2+, Mg2+, Mn2+, Al3+, Fe3+) and electrical conductivity.

Results: Soil concentrations showed a significantly positive relationship with egg concentrations and explained a large amount of variation in egg concentrations for the majority of PFAS ($R^2_{\text{partial}} = \pm 40\%$). Organic matter and the interaction $pH$:clay content showed a significantly negative and positive association with egg concentrations for abundant PFAS in eggs, respectively. For abundantly occurring PFAS in eggs (eg. PFOS; Fig. 1), robust and accurate prediction models could be developed with soil concentrations, OM and $pH$:clay content as predictors. Both internal cross-validation and external validation (newly collected egg data from 2022; see green cross symbols in Fig. 1) indicated reliable model performance. Adult earthworm PFOS and vegetable PFBA concentrations were associated with higher egg concentrations for the corresponding compounds, respectively.

Discussion and Conclusion: In agreement with existing literature data, soil sorption interactions probably play an important role in explaining PFAS bioavailability24. The reverse associations between egg concentrations and soil OM (-) and clay fractions (+) suggests that hydrophobic (predominant in OM) and electrostatic (predominant in clay fractions) interactions may, respectively, reduce and increase the bioavailability of PFAS, which warrants further elucidation in lab conditions. Based on these results, remediation measures can be formulated that potentially lower PFAS exposure via intake of homegrown eggs. The prediction models suggest that quicker and more cost-effective human risk assessment of PFAS exposure can be achieved.

Acknowledgments: The authors thank Tim Willems and Anne Cools for the assistance with the PFAS and soil parameter analyses and the FWO institute for the funding of the present research.

10:30 - 11:50

**Levels and Trends (Abiotic)**

*M.Venier & S.Yin*

**MON-AM-B1 Increasing Atmospheric Depositions of Trifluoroacetate (TFA) over the Last Decades**

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1. Introduction:

Trifluoroacetate (TFA), the anion of trifluoroacetic acid, is a very persistent and very mobile (vPvM) contaminant that occurs ubiquitously in the environment. Joudan et al. emphasized that there is insufficient scientific evidence supporting the existence of naturally formed TFA [1]. Therefore, according to current knowledge, the presence of TFA in the environment is solely the result of human activities. There are multiple anthropogenic sources of TFA such as industrial discharges as well as the degradation of various compounds containing a carbon-bound trifluoromethyl moiety (C−CF3), including many pesticides and pharmaceuticals [2]. Moreover, TFA can be formed in the atmosphere through the oxidation of certain perfluoroalkyl-containing substances, such as hydrofluorocarbons (HFCs), hydrochlorofluorocarbons (HCFCs), hydrofluorolefins (HFOs), and hydrochlorofluoroolelefins (HCFOs), which were introduced as substitutes for ozone-depleting chlorofluorocarbons (CFCs) and find wide application as refrigerants, fire extinguishing agents, and physical blowing agents [3]. Another source of TFA is the thermolysis of fluoropolymer products, such as polytetrafluoroethylene (PTFE) during their use or incineration [4]. In addition, specific fluorinated inhalation anesthetics (e.g. isoflurane) as well as various fluorotelomer compounds and perfluoroalkane sulfonamides can undergo atmospheric oxidation to produce TFA as well as other perfluorocarboxylic acids [5]. Because of its low Henry’s law constant and high water solubility, TFA is mainly scavenged from the atmosphere by wet deposition [3]. The usage of numerous gaseous precursors of TFA has considerably increased over the last decades. For instance, the atmospheric concentration of 1,1,1,2-tetrafluoroethane (HFC-134a) at Mauna Loa, Hawaii, USA, has gone up from ∼1.5 parts per trillion (ppt) in 1995 to ∼124 ppt in 2021 [6]. Therefore, with increasing emissions of these compounds, the TFA levels in precipitation are expected to rise.

Vascular plants can take up TFA by the transpiration stream or directly from rain and fog due to its hydrophilicity and small molecular size. Short-term laboratory experiments showed that TFA can be efficiently translocated within the organism and accumulate in the foliar tissue of plants [7]. However, so far, it is unknown to what extent TFA is accumulated in plants over longer periods (i.e., multiple years or decades) in natural ecosystems. The exposure of biota to TFA is a matter of concern due to the exceptionally high resistance of TFA to (bio)chemical degradation, its accumulation in certain environmental compartments, and the expected increase in TFA emissions. Human biomonitoring is already indicating widespread exposure to TFA. As such, TFA was found in 97% of serum samples from 252 Chinese adults in 2017 (detection limit: 0.12 µg/L), at a median concentration of 8.5 µg/L [8]. Despite using a relatively insensitive analytical method with a detection limit of 20 µg/L, TFA was still detected in urine in 30% of 83 Flemish adolescents in 2016/17 [9].

The EU Chemicals Strategy for Sustainability is one of the policy pillars of the European Zero Pollution Action Plan and aims *inter alia* to phase out the use of per- and polyfluoroalkyl substances (PFAS) for nonessential uses and to regulate these compounds as one group of chemicals [10]. In response, the authorities of several European countries agreed to develop a joint REACH restriction proposal by 2023 to limit the risks to the environment and human health from the manufacture and use of a wide range of PFAS [11]. PFAS include TFA under the Organisation for Economic Co-operation and Development (OECD) definition [12]. Temporally resolved exposure data are of special interest, as they can show the effect of the growing PFAS market on the environmental PFAS burden and allow for future predictions. So far, long-term trends for TFA in terrestrial ecosystems could not be included in the assessment due to lack of data. Therefore, archived plant samples from the German Environmental Specimen Bank (ESB) were analyzed for TFA to give insights into the levels, accumulation, and temporal trends of TFA in plants.

2. Materials and Methods:

Methanol (MeOH, > 99.9%) was obtained from Honeywell Riedel-de Haën (Seelze, Germany). Formic acid (> 98%) was procured from ACROS Organics (Geel, Belgium). The sodium salt of TFA (99.3%) and ammonium bicarbonate (> 99.5%) were obtained from Sigma-Aldrich (Steinheim, Germany). The isotopically labeled internal standard (IS) sodium TFA-13C2 was purchased from TRC (Toronto, Canada). Ultrapure water was provided by an arium pro laboratory water purification system (Sartorius AG, Göttingen, Germany).
In total, 110 tree leaf samples from the German ESB were analyzed for TFA. The sampling sites represent major ecosystem types in Germany with differing intensities of anthropogenic activity and land use. Samples were collected at the sites Berchtesgaden National Park (NP) (high mountainous region; near-natural ecosystem), Bavarian Forest NP (characteristic low mountain range in Central Europe with extensive forests; near-natural ecosystem), Harz NP (northernmost low mountain range in Germany, almost completely covered with forests; near-natural ecosystem), Solling (low mountain range, forestry ecosystem), Leipzig conurbation (region in the chemical triangle of Central Germany), and Saarland conurbation (formerly heavily industrialized conurbation in Southwest Germany). The study encompassed the species European beech (Fagus sylvatica, n=25), Lombardy poplar (Populus nigra 'Italica', n=24), Norway spruce (Picea abies, n=30), and Scots pine (Pinus sylvestris, n=31). Annual sampling for the species European beech and Lombardy poplar was conducted in late summer from August to mid-September before leaf discoloration. Leaves without petioles were collected. Samples of the coniferous species Norway spruce and Scots pine were gathered in spring from March to the end of May between snowmelt and new spring flushing. One-year-old shoots were used as the target matrix. To obtain high quality environmental data, every step in the procedure from sampling through transport, preparation, and storage is highly standardized within the German ESB.

For the extraction of TFA from plant matrices, 0.25 g of freeze-dried, homogenized, and finely ground plant material was weighed into a 15 mL polypropylene (PP) centrifuge tube, spiked with a defined amount of IS and mixed with 0.8 mL of MeOH and 0.8 mL of ultrapure water (+1% (v/v) formic acid). The obtained suspension was agitated for 15 min using a reciprocating shaker and sonicated for another 15 min. After centrifugation (15 min, 4700 g), the supernatant was transferred to another 15 mL PP centrifuge tube. This procedure was repeated twice with fresh extractant to optimize the extraction yield. Ion exchange liquid chromatography (IC; Agilent 1260 Infinity II LC system, Waldbronn, Germany) coupled to negative-ion electrospray tandem mass spectrometry (ESI-MS/MS; API 6500+ Q-Trap, Applied Biosystems/MDS Sciex Instruments, Concord, ON, Canada) was used for the analysis of TFA in the extracts (see [2] for a detailed description of the analytical method). The TFA concentrations in plants allowed for a 5-fold dilution of the combined extract with ultrapure water, leading to a reduction of matrix effects in subsequent IC-MS/MS-analysis.

3. Results:
The TFA concentrations of investigated deciduous and coniferous tree leaf samples generally ranged from tens to hundreds of µg/kg based on dry weight (dw). TFA concentrations in the European beech leaf samples ranged between 25 µg/kg dw (sampling year: 1989; sampling site: Berchtesgaden NP) and 310 µg/kg dw (2019; Solling). Considerably higher concentrations were found in leaves of the Lombardy poplar, ranging between 160 µg/kg dw (1991; Saarland conurbation) and 1100 µg/kg dw (2020; Saarland conurbation). Concentrations of TFA in the Norway spruce samples from the Saarland conurbation were between 58 µg/kg dw (1985) and 960 µg/kg dw (2021). Comparable concentrations (67 µg/kg dw (1992) to 760 µg/L kg dw (2022)) were found for the Scots pine samples collected at the Leipzig conurbation site. Samples from different locations of the same specimen (European beech; Lombardy poplar) were each in a similar concentration range (Figure 1).

Figure 1: Temporal concentration trends of TFA in leaf samples from various sampling sites in Germany.
Mann–Kendall trend tests were performed to assess if there was monotonic temporal trend in the TFA concentration in studied plants. Table 1 contains the Kendall’s tau (τ) correlation coefficient, the associated p-value, and slope of the Theil–Sen estimator. The latter provides information on the magnitude of the trend over the study period. With the exception of the Solling site, significant (p<0.05) positive trends in the TFA concentration were found for all of the deciduous and coniferous tree species. The slopes of the Theil–Sen estimator ranged between 4 and 24 (µg/kg dw)/a.

Table 1: Results of Mann-Kendall tests to identify temporal trends of TFA concentrations in studied plants. * determined by linear interpolation using the concentration values of the first and second sampling year since no concentration value of the year 1995 was available.

<table>
<thead>
<tr>
<th>Species</th>
<th>Sampling site</th>
<th>Sampling period</th>
<th>Kendall’s τ</th>
<th>p-value</th>
<th>Theil–Sen slope in (µg/kg dw)/a</th>
<th>Fold change in concentration between 1995 and 2018</th>
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</thead>
<tbody>
<tr>
<td>European beech</td>
<td></td>
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<td></td>
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<td>Berchtesgaden NP</td>
<td>1990–2018</td>
<td>0.90</td>
<td>0.0028</td>
<td>4.1</td>
<td>5.4*</td>
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<td>1989–2018</td>
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<td>0.0028</td>
<td>4.6</td>
<td>2.4*</td>
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<td>0.0302</td>
<td>7.6</td>
<td>3.2*</td>
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<tr>
<td>Solling</td>
<td>1999–2019</td>
<td>0.80</td>
<td>0.0833</td>
<td>7.7</td>
<td>N.D.</td>
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<td>Lombardy poplar</td>
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<tr>
<td>Leipzig conurbation</td>
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<td>0.67</td>
<td>0.0018</td>
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<td>Saarland conurbation</td>
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<td>0.0002</td>
<td>19</td>
<td>3.4</td>
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<td>Norway spruce</td>
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<td>&lt;0.001</td>
<td>15.4</td>
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<td>Scots Pine</td>
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<td>1992–2022</td>
<td>0.82</td>
<td>&lt;0.001</td>
<td>13.3</td>
<td>4.7</td>
</tr>
</tbody>
</table>

4. Discussion:

It can be assumed that the predominant source of TFA in the studied plant matrices was atmospheric deposition. Since TFA was detected in all analyzed plant samples, these results show the ubiquitous presence of TFA in dry and wet deposition even at near-natural and remote locations, such as Berchtesgaden NP. The determined levels of TFA in the latest tree leaf samples in Germany are comparable to the observations made by a recent study from China, which reported TFA concentrations between approx. 560 and 3000 µg/kg dw for similar matrices [13]. Benesch and Gustin found that TFA accumulated in ponderosa pine needles misted with environmentally relevant concentrations of TFA for a duration of four months. Relative to the TFA concentrations at the beginning of the experiment, concentrations of needles exposed to 0.15 and 10 µg/L of TFA increased by 10±5 µg/kg dw (n = 6) and 300±150 µg/kg dw (n = 6), respectively. No visual morphological (e.g. leaf shape, plant stature, dead leaves) or photosynthetic effects were observed (average TFA concentration in the leaf tissue at the end of the experiment: ∼320 µg/kg dw) [7]. To the best of our knowledge, no ecotoxicological effect data exist for deciduous and coniferous tree species at higher TFA levels, such as those reported here.

The increase in the TFA levels of studied plant samples within the last three decades (approx. 2- to 5-fold change between 1995 and 2018; Table 1) is in accordance with our previous study, which suggested that the average precipitation-weighted TFA concentration in precipitation in Germany increased by a factor of 3–4 within the period between 1995/1996 (average: ~0.1 µg/L) [14] and 2018/2019 (average: 0.34 µg/L) [15]. Pickard et al. also observed an upward trend in the atmospheric deposition of TFA in two Arctic ice cores starting around 1990, when the Montreal Protocol was introduced to phase out ozone-depleting CFCs with substitutes that oxidize to form TFA [16].

The uptake and transpiration of water by vascular plants to replace the water lost due to photosynthesis provides a mechanism for bioaccumulation of TFA. Transpiration is considered the main reason why hydrophilic PFAS accumulate especially in strongly transpiring plant parts, mainly the leaves [17]. Consequently, increasing levels of TFA in the studied perennial plants are likely a product of both phytoaccumulation over multiple years and an upsurge in the anthropogenic emissions of gaseous TFA precursors, leading to an increasing atmospheric deposition of TFA in the Northern Hemisphere over the last three decades.
MON-AM-B1  Increasing Atmospheric Depositions of Trifluoroacetate (TFA) over the Last Decades

Note that the earliest analyzed plant samples in the here presented study predate the introduction of HFCs in the early 1990s. Hence, TFA in samples pre-1990 is attributed to other sources, such as fluorotelomer and HCFC oxidation.

The elevated TFA concentration levels in samples of the Lombardy poplar, in comparison to samples of the European beech, could be due to differences in the transpiration rates of both species. Poplars are a fast-growing tree species and need substantially more water than other deciduous tree species, which could result in a higher potential for uptake and accumulation of TFA. For instance, the measured evapotranspiration per growing season (GS) of a hybrid poplar (Populus maximowiczii × P. nigra) plantation stand in Saxony, Germany, was considerably higher (622 mm/GS) than that of a European beech stand in the same state (386 mm/GS), although dissimilarities in the age of the stands and site conditions could also play a role in the differences in the evapotranspiration rates [18]. Another explanation for the generally higher concentrations in leaf samples from the Saarland and Leipzig conurbation sites could be the fact that both are located in more urbanized and industrialized regions in Germany. In our previous study we measured TFA in approx. 1200 precipitation samples collected over the course of 12 consecutive months in 2018/19 at eight sites across Germany. The three sites with the highest annual TFA wet deposition fluxes were all located in densely populated regions [15]. The phenomenon of urban enrichment of TFA has also been observed by other authors. For instance, TFA concentrations in surface waters around immediately downwind of urban areas in Northern California were about five to six times higher than in upwind areas [19].

All of the studied plant matrices show a more pronounced TFA increase in the later years of the study period (Figure 1). This is in accordance with the modeled exponential increase in the TFA emissions resulting from the atmospheric degradation of TFA-forming HCFCs, HFCs, HFOs, and HCFOs released in Europe (EU-28) after the year 2015. It was estimated that the TFA formation resulting from emissions of these precursors in Europe has strongly increased from 9 kilotons in 2015 to 15 kilotons in 2020 [3]. It is expected that the atmospheric formation and deposition of TFA will continue to grow in the coming years mainly due to rising global emissions of fluorinated refrigerants and also due to the switch to the latest generation of refrigerants, namely HFOs [3]. For instance, Holland et al. stated that the transition from HFC-134a to HFO-1234yf (assuming consumption is matched) would result in a 33-fold increase in the global atmospheric TFA burden, as the degradation of the latter occurs with a higher rate and with a larger TFA yield [20].

5. Conclusions:
This study has shown that the analysis of plant matrices can be an efficient biomonitoring tool to evaluate the temporal and spatial presence of TFA in the terrestrial environment. However, more analyses are needed to better understand the role of biomass loss on the TFA accumulation by plants and the fate of TFA from decomposing plant litter. Moreover, there is a need to better assess the human and environmental toxicity of TFA at chronic low dose exposures.

TFA will remain in the environment once released due to its exceptionally high persistence. With ongoing and increasing emissions of TFA from multiple anthropogenic sources, in combination with its high mobility in water and soil, high potential for long-range transport, as well as the absence of economically feasible remediation techniques, concentrations of TFA in terrestrial plants and other environmental compartments/matrices will increase. Therefore, in addition to continued monitoring of TFA in the environment, TFA and its precursors should be considered for regulation to minimize the risk of potentially irrevocable harm in the future.

6. Acknowledgments:
This work was funded by the German Environment Agency (Umweltbundesamt, UBA) (Project 157993 and 172963). We thank Gabriele Hoffmann and Jan Koschorreck from the UBA for their valuable support.

7. References:
MON-AM-B1 Increasing Atmospheric Depositions of Trifluoroacetate (TFA) over the Last Decades


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* Presenting author

Introduction:
In 2011 we established an Australian air monitoring program (i.e. the Passive Air (XAD) Monitoring and Archiving Network – abbreviated PAXMAN) that used passive air samplers originally developed by Umlauf and subsequently picked up calibrated and established by Wania and colleagues in the early 2000 (Wania et al 2003). These resin (XAD-2) filled samplers have a very high sorption capacity and relatively low surface area hence remain under typical environmental condition in a linear sampling phase periods well beyond a full year (Gouin et al. 2008). Note that with a ‘linear sampling phase’ we expect that clearance of analytes of interest from samplers is negligible and thus net accumulation during deployment is foremost a function of the concentration of the analyte in the air.

The PAXMAN program focused on establishing a minimum of 40 sites across Australia where between 2 and 4 samplers were set up and retrieved annually by local volunteers (typically employees of state government agencies). As the name of the program suggests the focus was on archiving samplers in anticipation that temporal changes in annual mean concentration of POPs is likely to change relatively slowly and naturally there will be a substantial level of uncertainty associated with such long-term passive sampling based monitoring approaches. Hence, we planned that a decade of sampling may provide some initial temporal data and thus we focused on sampling and archiving of samplers and did not analyse samplers. However, to assess that samplers and analytical techniques are sufficiently sensitive to be able to detect and quantify chemicals we undertook a small spatial study early in the program and established that we can measure a large set of POPs and other analytes such as pesticides and can clearly differentiate chemical ‘profiles’ that are associated with different land-uses (i.e. urban versus agriculture versus remote) (Wang et al. 2015).

Now that 10 years of sampling has been completed, we aimed to undertake a first evaluation of spatio-temporal trends of organic pollutants across Australia. The aim of this presentation is to provide insight into results from two components of the study, including 1) to evaluate the effectiveness of passive samplers for assessing spatio-temporal trends (sensitivity, reproducibility, any blank or other issues associated with the study design, and 2) to evaluate long-term trends of chemicals at three selected sites by analysing annual samples.

Materials and Methods:
Three sites belonging to three different land-use types and geographies were selected in this study. There were:
Site A: an agriculture, which was established in a rural town in South Australia’s Murray Darling agricultural area.
Site B: an urban/industrial site which is a major coastal city in Queensland.
Site C: a remote site from a very small reef island on the southern Great Barrier Reef, approximately 100 km offshore with only a small research station but no tourism.
A broad range of POPs and emerging contaminants were analyzed, including polychlorinated biphenyls (PCBs), polychlorinated naphthalenes (PCNs), organochlorine pesticides (OCPs), polybrominated diphenyl ethers (PBDEs), chlorinated paraffins (CPs), organophosphate flame retardants (OPFRs), polycyclic aromatic hydrocarbons (PAHs), selected novel brominated flame retardants (nBFRs), hexabromocyclododecane (HBCDD), tetrabromobisphenol A (TBBPA), and per- and poly-fluoroalkyl substances (PFAS). Isotope dilution methods were used for the analyses of selected chemicals, with detailed methods published elsewhere (He et al. 2017, Wang et al. 2015, 2019, Drage et al. 2019, Bogdal et al. 2015).

Results:
The reproducibility of the sampling methodology was assessed by analysing two different passive air samples, which were deployed at the same site and same period, in different batches. The difference between the calculated results (CV%) for the majority of analytes was acceptable, with the CV% less than 50%. The biggest variation between duplicates was found for TCEP (CV%=80%). These results suggested that the study design including the use of resin filled sampler are effective to evaluate trends of trace organic pollutants including selected POPs in the atmosphere.

Concentrations of chemicals and their temporal trends from the three selected sites are shown in Figure 2. In total, 80 out of 108 organic pollutants, belonging to 8 different chemical groups, were detected in at least one sample, including 84% of PCBs, 86% of PCNs, 88% of PBDEs, 72% of OCPs, 100% of CPs, 78% of OPFRs, 50% of nBFRs. HBCDD was not detected in any of the samples. The median concentrations of $\Sigma_{19}$PCBs (sum of PCB 11, 28, 52, 77, 81, 101, 105, 114, 118, 123, 126, 138, 153, 156, 157, 167, 169, 180, and 189), $\Sigma_{14}$PCNs (sum of PCN 13, 27, 28, 36, 48, 50, 52, 53, 66, 69, 72, 73, and 75), $\Sigma_{11}$OCPs (sum of HCB, o,p′-DDE, p,p′-DDE, o,p′-DDT, p,p′-DDT, o,p′-DDD, p,p′-DDD, ∼-HCH, ∼-HCH, trans-chlordane and cis-chlordane), $\Sigma_{7}$BDEs (sum of BDE 28, 47, 99, 100, 153, 154, and 183; note that BDE 209 was not included due to no detections in any samples), $\Sigma_{3}$CPs (sum of SCCPs, MCCPs, and LCCPs), $\Sigma_{9}$OPFRs (TCEP, TCIPP, TDCIPP, TPhP, TnP, TBP, TeHP, TCP and TBOEP), and $\Sigma_{11}$PAH (sum of acenaphthene, acenaphthylene, anthracene, benzo[a]anthracene, chrysene, triphenylene, fluoranthene, fluorene, naphthalene, phenanthrene, and pyrene) were 19 pg/m$^3$, 0.39 pg/m$^3$, 20 pg/m$^3$, 2.2 pg/m$^3$, 55 pg/m$^3$, 870 pg/m$^3$, and 5100 pg/m$^3$, respectively.

Discussion:
As expected, the data indicate that there are clear spatial differences between sites where the urban sites had significantly higher concentration for many chemicals including PCBs, PCNs, PBDEs while the agriculture site had higher concentrations of some OCPs. The remote sites had the lowest concentrations for most of the analytes.

For chemicals that had already been regulated or banned, decreasing trends were found in some sites, while the temporal and spatial trends for some in-use chemicals were affected by the local sources, resulting in sudden increases in certain period.

Both indicator and dioxin-like PCBs concentration in urban site showed a decreasing trend in 2011/2020, but an increasing trend of PCB 11. The PCB concentrations in both agriculture and remote areas were comparatively lower than in urban site and they did not show a clear increasing/decreasing trend. The concentration of both Σindicator PCBs and Σdioxin-like PCBs generally increased in all sites, except in one of the urban sites, which showed a decreasing trend after 2017. Overall, there was a decreasing trend of PCN congeners detected in the urban site, except some high concentrations reported during the period of 2011-2020. PCN concentrations at both agriculture and remote sites highly fluctuated in 2011/2020, showing some increasing trends in recent years, but their values were comparatively low compared with the urban site.
A clear decreasing trend of $\sum_{BDE}$ concentration, and thereby the PBDE congeners can be noted in the urban site from 2011 to 2020. Temporal trends of PBDEs in agriculture and remote site also followed a decreasing trend with some exceptions, but lower to that has been reported in urban site.

**Conclusions:**
The XAD-2 based passive air samplers could effectively reflect the levels of a range of organic pollutants in the atmosphere. In the 10-year PAXMAN study, we have observed decreasing trends during 2011 and 2020 for many traditional chemicals, which were associated with the global and local regulations on their use.

**Acknowledgments:**
This work was funded by the federal government’s Department of Agriculture and Water. We particularly like to thank Dr Sara Broomhall, Dr Eva Holt and Dr Bruce Gray for all support with the study.

**References:**
2. Drage D.S., et al. 2019. Serum measures of hexabromocyclododecane (HBCDD) and polybrominated diphenyl ethers (PBDEs) in reproductive-aged women in the United Kingdom, Environmental Research, 177: 108631.
MORNING BREAKOUT SESSIONS

MON-AM-B3  Assessment of potential pesticide contamination in different environmental matrices: study of transfers to local populations.

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2 UFR Sciences fondamentales et appliquées, University of Lorraine, Campus Bridoux, 57070 Metz, France

Introduction:
This project is dealing with the issue of the contamination of the air from phyto-pharmaceutical products (PPPs) and the evaluation of a potential exposure of residents from those contaminations. Here, residents from a vineyard area have been studied. Indeed, from the general population, residents living closed to farming area who are more prone to be contaminated by PPPs. This population group is very important because they will directly be impact by a potential transfer of products during applications by leeway and volatilization followed by transportation on short distances. Pinpoint those expositions of PPPs on residents is mandatory to demonstrate a potential impact of the agriculture on air contamination.

In this project, it was proposed to set up an experimental methodology including measurement of atmospheric and dust contaminations to demonstrate a potential residents’ exposure to crop protection products.

Materials and Methods:
Nine housing including one as “reference” near vineyards crops – to evaluate potential exposure of residents living near these crops – were chosen to perform dust and air sampling between March 2018 and December 2019 in Ergersheim (Bas-Rhin, FRANCE). In total 347 passive air samples (indoor and outdoor) and 127 dust samples were collected.

38 molecules were analyzed during this study including molecules used by vine-grower but also used in field crops. An analytical method coupling Pressurized Liquid extraction (PLE), pre-concentration by Thermal desorption (TD) and analysis by GC/MSMS was used for the extraction and quantification of pesticides in air and dust samples [1].

Results:
Important result of this study is that the potential exposure of residents was a bit more important during application of crop protection products period but also that this exposition was not totally over outside those periods. A lot of molecules were detected in air and dust samples including molecules specific to field crops and not only to viticulture. Indeed, the six most found molecules were cyprodinil (fungicide), diflufenicanil (herbicide), fenpropidin (fungicide), metamitron (herbicide) and prosulfocarb (herbicide), compounds mainly used in field crops and cyproconazole, a banned fungicide in France since June 2021 and therefore after the field campaign. Also, the resident chosen as “reference” showed inferior levels of contamination than other resident but still a positive exposition.

Discussion and Conclusion:
This study demonstrated that passive sampling seems to be a good methodology to access a potential exposure of residents to crop protection products. This easy, quiet and cheap sampling technic allowed a large-scale sampling with an easy residents’ participation. Also, it seems important to note that including dust sampling bring complementary information to air sampling. A regular sampling with a short time frame between two appears to be important if we want to identify an influence of contamination according to the year period. A choice of “reference” housings far away from fields is not necessarily mandatory. Indeed, it seems that housings not directly closed to fields or protected by other houses or corpses for instance, are sufficient to show a difference in terms of the level of exposure.

Acknowledgments:
Authors want to thank inhabitants who have authorized and participated to the collection of air and dust in their houses. SICAT Sarl is also gratefully acknowledged for providing SiC foam used in this study.

This work was supported by the PRIMEQUAL and ECOPHYTO programs. They were gratefully acknowledged for their financial support.

References:
Dioxin and furan (PCDDFs) are hazardous persistent organic pollutants (POPs) which could cause negative health effect. Due to the scarcity of land space in Taiwan, PCDDFs air emission sources may have greater health effect to residents. Thus, PCDDFs emission management is very crucial to secure residents’ health. In order to evaluate domestic ambient air PCDDFs concentration trend and identify domestic main emission sources, this project has monitored ambient air PCDDFs over a decade and established emission inventory updated each year since 1992.

PCDDFs emission inventory is estimated using emission factor and activity data. The emission of over 1000 stationary emission sources in Taiwan is calculated from the stack test result and annual raw material usage reported by the emission source owners. Mobile sources and fugitive emission are also included in this project. Ambient air PCDDFs monitoring has been conducted in 22 provinces of Taiwan since 1999. Sampling time is 72 hours and sampling sites are all in residential urban areas. The sampling and chemical analysis procedures of the ambient air monitoring task followed the standard method (NIEA A809 11B) released by National Institute of Environmental Analysis (NIEA) of Environmental Protection Administration of Taiwan (EPAT).

The calculated air anthropogenic PCDDFs emission amount is 43.07 g I-TEQ in 2021. The emission amount has decreased in the last two decades resulted by the PCDDFs emission standards promulgated by EPAT. The emission quantity reduction is mainly achieved by steelmaking industry and incinerator. The ambient air PCDDFs in the last two decades also have shown a gradually declining trend. Annual average concentration in 2022 is 0.021 pg I-TEQ/m³. The ambient air PCDDFs concentration in southern Taiwan is relatively higher. The reason may be the open burning of agricultural waste, the location of the main emission source and wind field. Seasonal change in concentration is significant, higher concentration in winter is observed. Ambient air PCDDFs concentration may also be affected by fire accident.

Domestic PCDDFs emission amount has been reduced by 91% since 1992. In 2021 the main stationary sources include steelmaking industry (e.g. sinter, EAF, etc.), boilers and incinerators. Previous studies have shown that secondary copper smelting is also a main source (Chen, 2004) but secondary copper smelting have a wide range of emission factor depends on the fugitive emission collection efficiency. The emission factor used in this study is calculated by the domestic stack corresponding to each emission source which is more accurate and specific. Ambient air PCDDFs concentration in 2022 is 50% lower than that in 2001. The monitoring results in the past 2 decades are similar to previous studies (Chang et al., 2003, Huang et al., 2011). The seasonal changing trend is also shown in previous studies (Huang et al., 2011, Mi et al., 2012). Technologies adopted for PCDDFs emission reduction include activated carbon powder injection, SCR and catalytic bag filter. Fast cooling devices are also commonly seen to avoid the PCDDFs de novo temperature window in steelmaking industry. According to stack test result, air emission of main sources has improved recently while small scale emission sources have shown a higher emission potential in concentration. Both waste wood boilers and cremation ovens have higher concentration due to batch operation characteristic. Furthermore, to achieve the goal of carbon neutral in future, wider utilization of SRF and waste derived material is expected. Thus, more stack tests and regulations regarding these high potential emission sources are needed for future PCDDFs emission management.

References:
1. Introduction:
Regional projects to support the global monitoring plan (GMP) component under the Stockholm Convention on Persistent Organic Pollutants (POPs) in developing country regions were coordinated by the United Nations Environment Programme (UNEP) and financed by the Global Environment Facility (GEF) since 2016 (UNEP, 2009; UNEP, 2014a; UNEP, 2014b; UNEP, 2014c). The GMP recommends the use of passive air samplers (PAS) equipped with polyurethane foam (PUF) disks as a simple and cost-effective tool to measure and assess atmospheric concentrations of POPs (UNEP, 2021). To obtain comparable data, the 42 participating countries were provided with up to 12 PAS and cleaned pre-conditioned PUFs to allow for quarterly sampling at one site in the respective country. The PUFs were exposed for two years and changed every three months between 2017 and 2019. The projects addressed 28 of the 31 POPs listed in either Annex A, B, or C of the Convention. Results of the projects have been published for organochlorine pesticides (OCPs), industrial chlorinated POPs (indPOPs), and brominated flame retardants (BFRs) (de Boer et al., 2023), dioxin-like POPs (dl-POPs) (Abad et al., 2022), and perfluoroalkyl substances (PFAS) (Camoiras Gonzalez et al., 2021). At Dioxin2022, we presented a spatial assessment for dl-POPs based on toxic equivalents (Fiedler et al., 2022).

Here we assess the amounts of 28 POPs or OP groups measured in 381 ambient air samples on a comparative basis for regional occurrence, i.e. within a UN region but also for the geographic location as latitude, and altitude.

2. Materials and Methods:
The preparation of the PUFs and the setup of the PAS/PUFs followed established procedures. After exposure, PUFs were shipped with express mail to the expert laboratories at VU University for analysis of OCPs, indPOPs, BFRs, CSIC for dl-POPs and OCPs, indPOPs, BFRs in Latin America, or Örebro University for PFAS. Chemical analysis including instrumentation, procedures, and quality control/quality assurance has been described elsewhere (Abad et al., 2022; Camoiras Gonzalez et al., 2021; de Boer et al., 2023). The amounts of POPs, including metabolites or defined congeners, are given in ng/PUF for the sum of the compounds.

Table 1 summarizes the samples obtained and assessed: N indicates northern and S southern hemisphere; the numbers thereafter indicate the latitude in degree; the number after letter A indicates a range of altitudes in meter (m). In total, 381 samples were assessed. Most samples were from 15 countries in Africa (149), followed by 104 samples from 11 countries in GRULAC (Group of Latin America and the Caribbean); the nine Pacific Islands countries (PAC) and the seven Asian countries 64 samples, each. Most samples were from 2018 and the majority of the sampling locations were located on the northern hemisphere (58%). 38% (144) of the sampling locations were around the equator at ±10° N or S latitude; thus, tropical climate conditions. 60% (229) of the sampling locations were at low altitudes (<200 m); but with Ethiopia in Africa and Ecuador in GRULAC, there were also two locations at very high altitudes (>2000 m) that contributed 20 samples.

All data were maintained in Microsoft Office 365 Excel®; visualization, statistical evaluations, and principal component analysis (PCA) were made using R (version 4.2.2) and R packages with R-Studio (2022.12.0 Build 353, 2009-2022 Posit Software, PBC).

Table 1: Number of samples analyzed by region, latitude, altitude or hemisphere

<table>
<thead>
<tr>
<th>Region</th>
<th>Africa</th>
<th>Asia</th>
<th>PAC</th>
<th>GRULAC</th>
<th>Overall</th>
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<tr>
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<td>64</td>
<td>64</td>
<td>104</td>
<td>381</td>
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<td>Sampling year</td>
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<td>70</td>
<td>36</td>
<td>35</td>
<td>53</td>
<td>194</td>
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<tr>
<td>Y2019</td>
<td>16</td>
<td>18</td>
<td>12</td>
<td>2</td>
<td>48</td>
</tr>
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</table>
**MON-AM-C1** Comparative overview on occurrence and distribution of POPs from air measurements in 42 countries

<table>
<thead>
<tr>
<th>Latitude</th>
<th>N_23+</th>
<th>N_10-23</th>
<th>Eq</th>
<th>S_10-23</th>
<th>S_23+</th>
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<tr>
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<td>0</td>
<td>29</td>
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<tr>
<td>Eq</td>
<td>79</td>
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<td>35</td>
<td>20</td>
<td>144</td>
</tr>
<tr>
<td>S_10-23</td>
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<table>
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<th>A_60-200</th>
<th>A_300-1000</th>
<th>A_1000-1999</th>
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<td>20</td>
<td>35</td>
<td>110</td>
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<td>A_300-1000</td>
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<td>A_1000-1999</td>
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<td>0</td>
<td>10</td>
<td>20</td>
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</table>

<table>
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<tr>
<th>Hemisphere</th>
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<tr>
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<td>54</td>
</tr>
<tr>
<td>Latitude</td>
<td>50</td>
<td>10</td>
</tr>
</tbody>
</table>

### 3. Results:
Since not all countries achieved to have eight exposures and not all POPs were analyzed in all regions; e.g., PBDE 209 and HBCD were not analyzed in GRULAC samples, the total number of results were 381 samples with the number of analytes shown in Table 2.

**Table 2: Number of results for each group of POPs and by region (* PBDE 209 and PBB153 not measured in GRULAC)**

<table>
<thead>
<tr>
<th></th>
<th>Initial OCPs</th>
<th>New OCPs</th>
<th>Ind.POPs</th>
<th>BFRs</th>
<th>dl-POPs</th>
<th>PFAS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>aldrin, dieldrin, endrin, chlordane, DDT, heptachlor, mirex</td>
<td>Toxaphene</td>
<td>α-HCH, β-HCH, lindane, endosulfan</td>
<td>PCB6, HCB, PeCBz, HCBd</td>
<td>PBDE, PBDE209, HBCD, PBB153</td>
<td>PCDD, PCDF, dl_PCB</td>
</tr>
<tr>
<td>Africa</td>
<td>113</td>
<td>113</td>
<td>113</td>
<td>113</td>
<td>113, 111, 113, 113</td>
<td>89</td>
</tr>
<tr>
<td>Asia</td>
<td>49</td>
<td>49</td>
<td>49</td>
<td>49</td>
<td>49, 48, 49, 49</td>
<td>30</td>
</tr>
<tr>
<td>GRULAC</td>
<td>83</td>
<td>21</td>
<td>83</td>
<td>83</td>
<td>82, 0*, 79, 82</td>
<td>53</td>
</tr>
<tr>
<td>PAC</td>
<td>49</td>
<td>49</td>
<td>49</td>
<td>49</td>
<td>49, 48, 49, 49</td>
<td>23</td>
</tr>
<tr>
<td>Total</td>
<td>294</td>
<td>232</td>
<td>294</td>
<td>294, 211*</td>
<td>293, 207, 290, 293</td>
<td>195</td>
</tr>
</tbody>
</table>

The quantitative results ranged across several orders of magnitude, often for the same POP. For almost all POPs, there were many results below the limit of detection; underlined by the median values below the limit of detection; across all samples for aldrin (not in GRULAC), endrin, toxaphene, b-HCH, PCDF (as sum of 10 congeners; not in Africa and GRULAC), PBB153, and PFHxS (not in GRULAC), see Figure 1,
Mon AM-C1  Comparative overview on occurrence and distribution of POPs from air measurements in 42 countries

Sixteen of the 27 maximum values were found in African countries, namely DDT (895 ng/PUF), heptachlor (8.2 ng/PUF), α-HCH (18.0 ng/PUF), β-HCH (104 ng/PUF), lindane (61 ng/PUF), endosulfan (140 ng/PUF), PCB6 (290 ng/PUF), PBDE (16 ng/PUF), PBDE209 (130 ng/PUF), PBB153 (0.87 ng/PUF), PCDF (0.6 ng/PUF), dl_PCB (20.7 ng/PUF), PFOS (36.0 ng/PUF), PFOA (3.2 ng/PUF), PFHxS (7.9 ng/PUF), and FOSA (1.9 ng/PUF). Asian countries had four maximum values as follows: chlordane (66 ng/PUF), HCB (27 ng/PUF), HCBD (334 ng/PUF), and HBCD (76 ng/PUF) as had GRULAC: aldrin (2.6 ng/PUF), endrin (3.1 ng/PUF), toxaphene (4.8 ng/PUF), and PeCBz (55 ng/PUF). The maximum values for mirex (1.9 ng/PUF) and PCDD (1.9 ng/PUF) were found in PAC. Figure 2 summarizes the statistical data as box whisker plots on a logarithmic scale. The dominance of the various POPs in the different regions can be seen.

Figure 2: Box whisker plots of POPs concentrations by region and colored as POP group (green= initial OCPs, dark green=Newly listed OCPs, salmon=BFRs, blue=dl-POPs, and plum=PFAS)
The principal component analysis (PCA) shows that for the first dimension (Dim1), the most impact was from the four PFAS (PFOS and PFHxS, both >20%, PFOA 19%, FOSA 14%), followed by PBDE 209 with 11%; all other POPs with less than 5%. For Dim2, endrin and β-HCH had 16%, followed by α-HCH and lindane with 12% and 10%, resp.; all other POPs had less than 10% contribution of the variables to the PCA. The biplot is shown in Figure 3. The outliers for Dim1 were the samples from Zambia and for Dim2 from Tanzania, both with black dots indicating Africa. The PCAs show that the African samples spread much wider than the other regions as shown by the wide concentration ellipse.

Figure 3: PCA biplots with concentration ellipses around the regions and the hemisphere.

4. Discussion:
The project has generated an abundance of data, which still needs to be assessed, especially by each country to prioritize actions under the Convention. Whereas the highest overall values and as mean and median value were found for DDT (31 ng/PUF; 12 ng/PUF), the mean and median values for some other POPs were relatively close together; for example: PCB6 (10.3 ng/PUF, 3.5 ng/PUF), dieldrin (6.2 ng/PUF, 2 ng/PUF), chlordane (5.1 ng/PUF, 1.7 ng/PUF), endosulfan (4.2 ng/PUF, 0.9 ng/PUF) or lindane (3.4 ng/PUF, 1.5 ng/PUF). Our data did not indicate any tendency with higher concentrations towards greater latitudes nor higher altitudes as shown in
MON-AM-C1  Comparative overview on occurrence and distribution of POPs from air measurements in 42 countries

**Figure 4:** Barplot displaying the median values of the POPs according to latitude (above) and altitude (below).

5. Conclusions:
With two years of sampling and selective and sensitive POPs analysis, a large number of data has been generated. The data have been used for global interpretation but at national level, these results will serve to prioritize sampling and analytes in future monitoring programmes.

6. Acknowledgments:
The project was founded by UNEP with funds provided by the Global Environment Facility (GEF, Washington, DC, USA). We thank the national coordinators in the 42 countries for their dedicated work in implementing the PAS/PUF component of the projects.

7. References:
Introduction: FR are compounds applied to consumer products and materials to slow down or to hinder their combustion (de Wit, 2002), being useful to improve the life cycle of products (Hahladakis et al., 2018). During the last years BFRs have been progressively regulated (eliminate production and use) by the Stockholm Convention and implemented by the European Commission (EU Regulation, 2017). There are about 75 nFRs that have been manufactured, for which their production volume, high distribution in the environment, bioaccumulation, and toxic potential position them as priority contaminants (McGrath et al., 2017). The BioBio River basin is located in central Chile and flows 380 km to the Gulf of Arauco (Golfo de Arauco) located in the VIII Region or Región del BioBio, where it discharges into the Pacific Ocean. The objectives of our study are to investigate the PBDE contamination levels and spatial and seasonal distributions of PBDEs in water along the BioBio River.

Materials and Methods: The sampling campaign was conducted in central Chile along the Biobío River (36°26’S; 38°29’S - 72°10’W; 73°40’W) and Itata river (36°23’S - 72°51’O) in January 2022. With a total of 13 sampling sites, the sampling zone was divided as rivers of the Upper area, rivers of the Middle area and rivers of the Lower area. Water samples were analyze as reported previously in Pozo et al., 2022.

Results: Levels (ng/L) of PBDE 209 accounted almost 98% of total PBDEs composition and were as follow: Upper zone (0.04-0.86), Middle (0.08-0.64), and Lower (0.03-1.22). The lower area of the river (Mouth of Biobio River) showed the heist concentration. In case of DPs the values were for Upper zone (0.03-1.25), Middle (0.15-0.99), lower (0.11-2.95) and accounted for 60% Syn-DP and Anty-DP (40%). These results continue with the most recent knowledge of Pops in te BioBio River in central Chile. Further research is till ongoing.

Discussion and Conclusion: Levels in this study are lower compared with other areas in the world. Cui et al., (2019) reported ∑PBDEs the main river under study in the city of Wuhan with 0.88 and 1.53 ng L-1 in the winter and summer seasons respectively. Also, Liang et al. (2019), la concentration de ∑9 PBDE in the Guanlan River (average 115,72 ng/L for dry season and 22,15 ng/L during wet season).

Acknowledgments: This study was funded project Fondecyt 1211931 (KP). The authors thank the RECETOX RI (No LM2023069) financed by the Ministry of Education, Youth and Sports of the Czech Republic, and Operational Programme Research, Development, and Education – project CETOCOEN EXCELLENCE (No CZ.02.1.01/0.0/0.0/17_043/0009632) for supportive background.

References:
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5Department of Chemistry, University of Sciences, Hue University, Hue 530000, Vietnam

Introduction:
In May 2015, 75 congeners of polychlorinated naphthalenes (PCNs) were included as new POPs under Stockholm Convention. PCNs production started in the early 1900s and widely adopted in a variety of industrial applications such as dye-making, fungicides in the wood, textile, and paper industries, plasticizers, oil additives, casting materials for alloys, and lubricants for graphite electrodes. Although the production of PCNs was terminated at the end of the 20th century due to the recognition of their impact on the environment and adverse effects on human health, these compounds are still released into the environment by common sources such as historical use of PCNs, historical and present use of PCBs (PCNs are byproducts in the production of PCBs), combustion and other thermal processes. The level of PCNs in the environment of Vietnam has been not reported 1.

Ho Chi Minh City (HCMC) is the most densely populated city and considered the biggest economic center in Vietnam which is facing a serious air pollution problem. Moreover, several industrial zones located in and around the city might potentially emit PCNs. As the first study on PCNs in ambient air of HCMC and the compliance of Vietnam’s government on Stockholm convention, investigation of PCNs characteristics including level, potential source, and health assessment is necessary. In this study, ambient air samples were collected using high-volume air samplers at five urban sites (UTE, UOS, BT, PN, and NB) during the dry season (from March to May 2018) and two sites (UTE and UOS) during the rainy season (from August to October 2018) in Ho Chi Minh City (Fig. 1). As the very first study on PCNs in Vietnam, investigating the level and exploring potential sources of PCNs in ambient air in Ho Chi Minh City (HCMC) which is one of the densest populations with many potential sources of PCNs within and around the city is deemed necessary.

Materials and method:
In this study, ambient air samples were collected using high-volume air samplers (PS1, USA) at five urban sites. Each sample was collected for 2 days with a total volume of 650 m³. Gas-phase PCNs were adsorbed by a pre-cleaned sandwich cartridge of PUF/XAD-2/PUF containing 15 g XAD-2 and a PUF (Tisch Environmental), while particulate-phase PCNs were collected by quartz fiber filter (QFF, Whatman). Three samples were collected from each site for dry and rainy seasons; 75 PCNs in gas and particulate phases were analyzed using GC/MS following isotope dilution method 2. Procedures of pretreatment and analysis of PCNs were clearly presented in previous study 1. Briefly, samples were extracted for 24 h using Soxhlet extraction with dichloromethane (DCM) after spiking with 1 ng labeled 13C10 PCNs (Cambridge Isotope Laboratories, Inc., Canada) to quantify the concentration of each PCN congener.
The DCM extract was then concentrated and cleaned up using a multi-silica column coupled with a mini-carbon column (CAPE, Poland). Then, the collected eluent was re-concentrated to approximately 200 µL with a gentle nitrogen stream and added recovery standards (13C10-CN64) before analysis using HRGC/LRLM (Agilent 6890-5973N) with a fused silica capillary column DB-5 MS (60 m × 0.25 mm × 0.25 m) under positive EI conditions, and data were obtained in the selected ion monitoring (SIM) mode.

Results and Discussion:
The prevailing wind directions recorded during dry and rainy seasons were northeast and southwest, respectively. The temperature ranged from 26 – 40.3ºC (dry season) and 24.5 – 35ºC (rainy season), relative humidity ranged from 30 – 81% (dry season) and 50 – 98% (rainy season). Meanwhile, wind speed fluctuated between 0 – 4.5 m/s (dry season) and 1.5 - 7.1 m/s (rainy season), and measured dust concentration of TSP ranged from 41.1-94.8 µg/m³ (dry season) and 40.6 – 112 µg/m³ (rainy season). During the sampling period, the meteorological parameters have statistical differences between the two seasons (p-value < 0.05); however, the concentration of TSP dust has no statistical difference between the two seasons (p-value = 0.413 > 0.05). The rainfall recorded during the sampling period in the two seasons also differed slightly, suggesting that wet deposition may not affect TSP levels during the wet season.

The concentration of PCNs and correlations between PCNs and meteorological parameters are depicted in Fig. 2. In particular, the average total PCN concentration was 130.0 - 67.4 pg/m³ (dry season) and 88.2 - 61.1 pg/m³ (rainy season). The average PCN concentration was significantly greater in the dry season than in the rainy season (Fig. 2a). The total concentration of PCNs measured at the OUS was the highest and ranged from 184.4 to 263.7 pg/m³, followed by NB with the second highest concentration (151.7 - 211.5 pg/m³), then BT (88.9 - 166.7 pg/m³), UTE (71.4 - 72.3 pg/m³), and finally the lowest sample in PN (56.8 – 62.0 pg/m³). There was no statistically significant difference in the overall concentration of PCNs between UTE and PN sites (p-value >0.05). The wide variety of PCNs at OUS and NB sites may come from several sources. This can be explained by the fact that petroleum production facilities and petroleum depots tend to emit higher PCNs in the urban site (OUS) and the site close to industrial zone (NB).

The results also indicated that concentration of PCNs measured in this study during the dry season was mainly in the gas phase, accounting for only 0.02–0.22% of the total concentration. While the portion of particulate PCNs in the rainy season increased to 4.20 – 16.12%. This result is similar to those of. in Shanghai, China (gas phase accounts for 80.6 - 97.7%) and in Taiwan Taoyuan (97% gas phase). In Fig. 2b the particulate PCNs were positively correlated with wind speed and humidity (p < 0.05). In the rainy season, high humidity, low temperature, and higher wind speed than in the dry season may have led to higher particulate PCNs in the rainy season than in the dry season. However, PCNs concentration in gas phase or total PCNs concentration was not affected by wind speed, humidity, temperature, TSP dust concentration. In addition, higher temperatures in the dry season increased the evaporation of PCNs from evaporating sources such as products containing PCNs, which increases the concentration of PCNs, which may be the main reason for higher total PCNs concentrations measured in the dry season compared with those observed in the rainy season.

![Fig. 2. PCN concentration (a) and correlation of PCNs and meteorological parameters (b)](image-url)
MON-AM-C3  Occurrences of PCNs in ambient air of Polychlorinated Naphthalenes in Ho Chi Minh City, Vietnam

Based on the characteristics of homologous distribution of PCNs in samples at 5 sampling sites, they can be divided into 2 groups (Fig. 3). Group 1 includes OUS and NB samples with the contribution of TetraCN > TriCN > MonoCN > DiCN, which implies that PCNs collected from these sites can be emitted mainly from volatile sources from technical mixtures containing PCNs. Group 2 includes samples taken from UTE, BT and PN sites with a high contribution of light-molecular-weight homologs with 1 to 3 chlorine atoms (MonoCN, DiCN, TriCN), suggesting PCNs emission sources related to combustion processes or solid waste incineration after air pollution control devices.

In order to identify possible sources of PCNs collected in HCMC, diagnostic ratios which were developed in the previous study were used. In the dry season, two diagnostic ratios (CN45/36)/CN42, CN54/(CN53/55) also divide the samples into 2 groups. Group 1 consisted of samples taken at the OUS and NB sites with ratios (CN45/36)/CN42, CN54/(CN53/55) <1 and <0.8, respectively, indicating that PCNs were collected at these sites related to evaporation from products containing PCNs. While the values of these ratios in group 2 (UTE, BT, PN) are greater than 1 and 0.8, respectively, indicating that the PCNs obtained are related to combustion sources. The ratios (CN66/67)/(CN71/72) and (CN73)/(CN74) calculated for the two groups are greater than 2.5 and 0.4, respectively, indicating that the 6 and 7 chlorine homologues were typically emitted from combustion sources. However, the ratio of combustion source-related PCNs to PCNs was less than 0.11 in almost all locations, indicating that PCNs in these samples are not from combustion sources. Therefore, PCNs collected in the dry season were more likely attributed to the evaporation of PCNs from the use of PCN-containing products rather than to combustion sources or industrial processes utilizing combustion processes.

Conclusions:
In this study, 75 PCNs congeners of 21 ambient air samples including particulate and gas phases were collected with a high-volume sampler at 5 different sites in Ho Chi Minh City (HCMC) during the dry and rainy seasons and analyzed using the isotope dilution method and gas chromatography coupled with mass spectrometry (GC/MS). TSP concentrations ranged from 40.6 to 112 pg/m³ (54.1 16,46 g/m³) and were greater during the dry season than in the wet season. Five sites had PCNs concentrations ranging from 60.04 to 264 pg/m³. The PCNs measured in the gas phase were significantly greater than those in the particulate phase and accounted for the majority of the total PCN concentrations; However, the contribution of particulate PCNs was higher during the rainy season than during the dry season. The analysis of PCNs homologues revealed that group 1 consisting of OUS and NB sites received the largest contribution from TetraCN, followed by TriCN, MonoCN, and DiCN homologue; Group 2 consists of UTE, BT, and PN sites and has a greater contribution from homologs with 1 to 3 chlorine atoms (MonoCN, DiCN, TriCN). Evaporation of PCNs from technical mixtures containing PCNs might be the main source of ambient PCNs in Ho Chi Minh City.

Acknowledgments:
This research is funded by Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number 104.04-2019.35.

References:
MON-AM-C4  Determination of the Contamination by Environmental Pollutants in the Population of Kinshasa, Democratic Republic of Congo.

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Introduction:
Environmental contamination by Persistent Organic Pollutants (POPs) such as dichlorodiphenyltrichloroethane (DDT) and associated endocrine disruption is a worldwide public health concern. Many Western or Asian countries have initiated large scale studies at the regional or national level to investigate the background contamination of the population to these POPs in order to implement legislation to reduce exposure and to assess associated health risks. On the other hand, almost no data are currently available for sub-Saharan African countries. However, this biomonitoring is all the more important as DDT was banned in many African countries decades after it was banned in the Western countries. For example, DDT was banned in the Democratic Republic of Congo (DRC) in 2011, where it was previously used extensively to control mosquitoes that transmit malaria. The lack of environmental legislation in the DRC also raises concerns about other environmental pollutants, such as polychlorobiphenyls (PCBs) or heavy metals. The aim of the present study is thus to explore the exposure of the population of Kinshasa (capital city of the DRC) to DDT, PCBs and metals.

Materials and Methods:
151 individuals were recruited in Kinshasa during the last trimester of 2022 (95 men and 56 women, aged from 18 to 80 years) and were invited to provide a urine sample and two blood tubes (one was centrifuged to collect serum). 4,4'-DDE (the main metabolite of DDT), PCBs 28, -105, -114, -118, -138, -153, -156, -157, -167, -180 were measured in serum. Briefly, organochlorine compounds were extracted with a liquid-liquid extraction. The organic phase was purified on a phospholipid removal cartridge and then concentrated by evaporation before the analysis on a gas chromatograph coupled to a triple quadrupole mass spectrometer. Metals (Pb in blood and As in urine) were analyzed on an Inductively Coupled Plasma Mass Spectrometer.

Results:
The pollutant concentrations measured in the population of Kinshasa are gathered in the following table:

<table>
<thead>
<tr>
<th>Pollutant</th>
<th>Detection frequency</th>
<th>Median</th>
<th>Min-Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>4,4'-DDE</td>
<td>100%</td>
<td>828 pg/mL</td>
<td>34.7 - 40279 pg/mL</td>
</tr>
<tr>
<td>PCB 138</td>
<td>98%</td>
<td>6.8 pg/mL</td>
<td>&lt; 6 – 330 pg/mL</td>
</tr>
<tr>
<td>PCB 153</td>
<td>98%</td>
<td>37.3 pg/mL</td>
<td>&lt; 7 – 465 pg/mL</td>
</tr>
<tr>
<td>PCB 180</td>
<td>99%</td>
<td>50.6 pg/mL</td>
<td>&lt; 5 – 299 pg/mL</td>
</tr>
<tr>
<td>Pb</td>
<td>100%</td>
<td>54.9 µg/L</td>
<td>14.7 – 232 µg/L</td>
</tr>
<tr>
<td>As</td>
<td>100%</td>
<td>48.1 µg/L</td>
<td>2.9 - 372 µg/L</td>
</tr>
</tbody>
</table>

Discussion and Conclusion:
The present work is the result of a collaboration between Belgian and Congolese institutions, therefore, we compared the contamination determined in the population of Kinshasa with those measured in different biomonitoring studies carried out within the Belgian population. On one hand, the PCBs contamination observed in the Congolese population is much lower than those determined in the Belgian population in 2015 (median concentrations for PCB 153: 37.3 pg/mL vs 360 pg/mL [1]). On the other hand, as expected the levels of 4,4'-DDE were higher in the present cohort (median: 828 pg/mL vs 410 pg/mL [1]). We also observed higher levels of lead in the present population compared to the Belgian cohort (median: 54.9 µg/L vs 23.1 µg/L) [2]. This first biomonitoring study conducted in the DRC highlights the high exposure of the local population to DDT and metals and calls on us to investigate the health effects of this important contamination.

References:
MON-AM-D1  Regulated PFAS in Drinking Water and Food According to New EU Regulations –
A Streamlined Sample Preparation

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Introduction: Per- and polyfluorinated alkyl substances (PFAS) get into the environment during their manufacturing process and further also during their use and disposal. Analyzing these PFAS compounds is challenging and dedicated lab equipment helps to avoid blind values and standardizes the processes in sample preparation. This year, the EU Commission issued new, demanding guidelines (EU 2022/2388, (EU) 2022-1428) for the regulation and detection of PFAS in food. The new EU Drinking Water Directive (EU 2020/2184) also came into force this year. The requirements for the sensitivity of the method for demonstrating the limit values to be complied with are increasing all the time. A limit value of 0.1 µg/L for the sum of 20 PFAS is specified in the EU drinking water directive. The maximum permitted limit values for PFOS, PFOA, PFNA, PFHxS in animal foods are in the range of 0.3 to 1 µg/kg. Future limit values for an extended range of analytes in a wide variety of foods could even be significantly lower ((EU) 2022-1431). Achieving the LOQs within the framework of the methods for determining the limit values is therefore becoming increasingly difficult. Especially in matrices with a high number of impurities, detection is all the more difficult without prior purification using SPE. A blank value-free sample preparation with additional SPE is becoming more and more essential. The challenge is to develop a streamlined process to reach current and future reporting limits in a sufficient and reliable manner.

Materials and Methods: The full workflow of PFAS sample preparation is presented. Analyte extractions of solid samples via different methods were conducted. For the enrichment and/or purification of PFAS compounds solid phase extraction (SPE) with newly developed SPE cartridges were applied. For the critical evaporation step a vacuum centrifuge with cold trap named D-EVA was used. The samples were subsequently analysed by LC-MS/MS.

Results: The used blind value free robotic system FREESTYLE in combination with the newly developed SPE cartridges shows excellent reproducibility, high recoveries and low standard deviations. The right evaporation method ensures no loss of volatile and long chain PFAS in the final sample preparation step.

Discussion and Conclusion: The presented workflow shows a streamlined sample preparation process with extraction, automated sample preparation for PFAS analysis, which can be used for different kinds of PFAS including neutral sulfonamides and long chain analytes in drinking water and food matrices. Further, SPE cartridges with a superior performance for enrichment and clean-up of PFAS from drinking water and food matrices were developed.
Progress in Methods for POPs Analysis

W. Tirler & G. Eppe

Assessing the performance of high resolution ion mobility-mass spectrometry for the separation of GC coeluting isomeric and isobaric halogenated persistent organic pollutants

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Introduction:
POP1s, or persistent organic pollutants, are industrial chemicals that are toxic, persistent, and bioaccumulative, and can be released into the environment intentionally or unintentionally1. They are a cause for global concern, as they pose risks to both human health and wildlife. The Stockholm Convention was implemented in 2004 to address the anthropogenic release of these contaminants, and it currently regulates more than 29 harmful substances2. However, there are also new emerging persistent and bioaccumulative organic compounds that are not yet regulated and discovered in increasing numbers in the environment3. To monitor halogenated POP1s in various types of samples, standard protocols typically involve long sample preparation procedures followed by mass spectrometric analysis, often preceded by chromatographic separations4,5. Because different classes of POP1s exhibit a wide range of physicochemical properties, a combination of liquid chromatography (LC) and gas chromatography (GC) separations is typically required, with GC being the preferred separation method for analytes that are (semi)volatile, non-polar, and thermally stable4. However, detecting and measuring POP1s in complex samples using chromatographic-based MS analysis still faces major limitations due to the presence of isobars and isomers that coelute in the chromatographic dimension. Indeed, isobars have the same nominal mass, and distinguishing between them therefore usually requires very high mass resolving power or tandem MS. On the other hand, coeluting isomers cannot be differentiated if they exhibit identical or similar fragmentation mass spectra7. This problem is particularly challenging for halogenated POP1s, which are characterized by a wide range of isomeric and isobaric congeners.

To overcome this limitation, enhanced selectivity has been achieved by using additional chromatographic dimensions such as GC×GC8. Alternatively, another approach to increase selectivity is ion mobility spectrometry9 (IMS), a highly effective separation technique that has recently shown great potential for analyzing small molecules such as drugs10, biological compounds11, and chemical pollutants12. Ion mobility spectrometry is a technique that involves separating ions in an electric field in the presence of a buffer gas13. In this process, ions are accelerated by the electric force but slowed down by multiple collisions with the gas, ultimately reaching a stationary state where they travel at a constant velocity, or drift speed. At a given electric field strength and charge state, the drift speed of an ion is proportional to a constant called the ion mobility constant (K). Small and compact ions experience fewer collisions with the buffer gas than large and extended ions, resulting in faster drift speeds. They are therefore characterized by larger ion mobility constants. In summary, ion mobility separates ions characterized by different ion mobility constants based on their drift speeds. Consequently, this technique offers an additional "orthogonal" dimension of separation to mass-to-charge ratio and retention times, providing additional selectivity13. Moreover, experimentally measured ion mobility constants K can be converted to a parameter known as the collision cross-section (CCS), which from a simplified perspective characterizes the ion gas phase size. This parameter can serve as an additional ion descriptor for ion identification, by comparing it with established values in databases or theoretically predicted values, offering further confidence in analyte annotation in both targeted and untargeted approaches13.

Recent studies have demonstrated the relevance of using ion mobility for pollutants screening, including several classes of halogenated persistent organic pollutants, such as PFAS14,15, PCBs16, PBDEs17,18, and chemical pollutants19. Ion mobility spectrometry is a technique that involves separating ions in an electric field in the presence of a buffer gas. In this process, ions are accelerated by the electric force but slowed down by multiple collisions with the gas, ultimately reaching a stationary state where they travel at a constant velocity, or drift speed. At a given electric field strength and charge state, the drift speed of an ion is proportional to a constant called the ion mobility constant (K). Small and compact ions experience fewer collisions with the buffer gas than large and extended ions, resulting in faster drift speeds. They are therefore characterized by larger ion mobility constants. In summary, ion mobility separates ions characterized by different ion mobility constants based on their drift speeds. Consequently, this technique offers an additional "orthogonal" dimension of separation to mass-to-charge ratio and retention times, providing additional selectivity. Moreover, experimentally measured ion mobility constants K can be converted to a parameter known as the collision cross-section (CCS), which from a simplified perspective characterizes the ion gas phase size. This parameter can serve as an additional ion descriptor for ion identification, by comparing it with established values in databases or theoretically predicted values, offering further confidence in analyte annotation in both targeted and untargeted approaches.

In this short paper, we delve deeper into the potential of ion mobility as a tool for separating isobaric and isomeric species that remain unresolved in the chromatographic dimension. While previous studies have demonstrated the ability of ion mobility to separate such species in direct infusion IM-MS applications17,19, they also highlighted the limitations of this technique for contaminants identification, including the reduction of false positive identifications and the classification of unknowns according to specific trendlines in the CCS vs m/z space.

For that purpose, we conducted an analysis on a complex standard mixture containing 175 persistent organic pollutants (POPs) using gas chromatography-mass spectrometry (GC-MS) coupled with a high-resolution trapped ion mobility spectrometry (TIMS) system. We discuss the advantages, limitations and prospects of ion mobility in this regard.
MON-AM-D2  Assessing the performance of high resolution ion mobility-mass spectrometry for the separation of GC coeluting isomeric and isobaric halogenated persistent organic pollutants

Materials and Methods:
A solution of 175 individual POPs was prepared by mixing standard solutions of halogenated dioxins (PCDD/Fs, PBDD/Fs and PXDD/Fs), biphenyls (PCBs, PBBs and PXBs) and diphenyl ether (PBDEs). Standards were purchased from Wellington (Ontario, CA) and CIL (Tewksbury, MA). The final concentration was 5-20 pg/µl in n-nonane (99%, Alfa Aesar).

The mixture of POP standards was analysed on a commercial timsTOF Pro II mass spectrometer (Bruker, Bremen) equipped with a GC-APCI source for sample introduction and ionization (GC-APCI II, Bruker, Bremen). Injections were performed in splitless mode (injection temperature 275°C, 1 ml) on a Bruker 456-GC equipped with a Rxi-5Sil MS column (30 m x 0.25 mm x 0.25 mm, Restek). A fast, linear GC oven temperature program was set in order to promote the coelution of isomers and isobars: 1 min 140°C, 10°C/min to 310°C, hold 20 min, for a total analysis time of 38 minutes. Helium was used as the GC carrier gas at a flow rate of 2 mL/min. APCI of the analytes was performed in positive mode and generated mostly stable radical molecular ions M+, along with a smaller fraction of protonated [M+H]+ ions. TIMS separations were performed in high resolution mode (R_p(avg) ~140, scan rate β, 40 V/s). The analysis were also performed in very high resolution mode, using an analysis range focusing only on some specific coeluting isomeric pairs (β, 10 V/s).

Results:
Under the short GC run conditions used in this study, although the majority of pollutants were fully resolved in the GC and/or m/z dimension (~78%), we observed a considerable fraction of coeluting or partially coeluting isobars and isomers (~10% and ~12%, respectively). One notable example of such coelution challenges was observed with the partially coeluting isomeric PeCBs (84, 90 & 101, m/z = 325.8799) and the isobaric DiBDF (2,4-DiBDF, m/z = 325.8760), as shown in Figure 1a. The small mass difference (∆m/z = 3.9x10^{-3}) between these species required a mass resolving power (m/∆m) on the order of 84,000 to achieve complete resolution in the m/z dimension. However, the resolving power of the TOF spectrometer used in this study (~50,000) was not sufficient to achieve this separation. Consequently, none of the four species could be fully resolved in either the GC or the m/z dimension in this specific case.

Figure 1 - (A) GC chromatogram of pentachloro biphenyls (PeCBs) and dibromodibenzofuran (DiBDFs) highlighting the (partial) coelution of PeCBs 84, 90, 101 (isomers) and 2,4-DiBDF (isobar). (B) Corresponding ion mobility spectrum (R_p = Resolving power).
However, the ion mobility dimension provided additional separation, as depicted in Figure 1b. The radical M⁺ ion of the dibromo-substituted furan was clearly distinguished from the three isomeric pentachloro substituted biphenyls due to the notable difference in CCS (>7%). For the three isomeric PCBs, baseline separation was achieved between the partially coeluting PeCB 84 and PeCBs 90-101. However, no distinction was noted for the latter perfectly coeluting isomeric pair (PeCBs 90 and 101).

Overall, we observed that all the (partially) coeluting isobaric species could be baseline separated in the ion mobility dimension, with a percent difference in CCS ranging between 2 and 8%. However, for the majority of the (partially) coeluting isomeric pairs, the corresponding ion mobility spectra were characterized by a single, convoluted peak. Indeed, among the (partially) coeluting PCB isomeric pairs observed in the study, only two cases (HxCB 128-167 and PeCBs 84-91/101) showed significant separation in the ion mobility dimension.

In order to gain a deeper understanding of the required resolving power to separate these challenging coeluting isomeric species, each of them were analysed separately in very high resolving power mode. The increased separation power led to partial separation of the PCBs 28-31 pair, with approximately 25% separation achieved between the two isomers (Figure 2a). Based on this result, the percent difference in CCS was calculated to be no greater than 0.6%. Similar improvements in separation, although less pronounced, were also noted for the isomeric pairs TCBs 66-70 and HxCB 153-168, with an estimated difference in CCS below 0.5%. However, for most of the (partially) coeluting isomers, no further improvement in separation was observed despite the increased separation efficiency (e.g., HxBDD isomers in Figure 2b).

Discussion:
The results presented above provide compelling evidence of the added value of ion mobility as an extra separation dimension for crucial isobaric pairs that co-elute in the chromatographic dimension. The observed percent difference in CCS, ranging from 2.0 to 8.0%, indicates that despite having almost identical mass, these isobars possess distinct chemical structures due to variations in the central aromatic moiety, type and number of halogen atoms. For example, the 2,4-DiBDF in Figure 1 is made of a dibenzodioxin core with two bromine atoms, while the PeCBs consist of a biphenyl core substituted with five chlorine atoms. Here, the DiBDF’s significantly lower CCS can be attributed to its much lower halogenation degree compared to the PeCBs20.
MORNING BREAKOUT SESSIONS MONDAY 11 SEPTEMBER 2023

Progress in Methods for POPs Analysis
W. Tirler & G. Eppe

MON-AM-D2 Assessing the performance of high resolution ion mobility-mass spectrometry for the separation of GC coeluting isomeric and isobaric halogenated persistent organic pollutants

As for the great majority of the coeluting isomers, however, our results indicate that their percent difference in CCS are too close (≤ 0.6 %) to enable their ion mobility separation, even with the higher resolving power afforded by the TIMS compared to other common commercially available IM platform, such as the linear TWIMS and DTIMS. These small differences in CCS are not surprising since all these isomers are positional isomers that only differ in structure by the position of 1 or 2 halogen atoms.

Interestingly, for the only two cases for which significant separation of the isomers was noted (PeCBs 84-91/101 in Figure 1b and HxCB 128-167), the respective PCB isomers had unequal extent of substitution at the ortho positions, unlike all the other (partially) coeluting PCB pairs which had the same number of chlorine substituents in those positions. This is consistent with observations made in the literature that PCB isomers exhibit lower collision cross section values as the extent of substitution at the ortho position increases. The trend is likely due to the adoption of a more compact conformation by non-coplanar PCBs, with chlorine substituents in ortho positions, compared to coplanar, dioxin-like PCBs having a higher extent of chlorine atoms in the meta and para positions.

This work demonstrated the potential of ion mobility as an additional dimension of separation to deal with the challenging coelutions of isomeric and isobaric species. While the coeluting isobars were structurally different enough to be baseline separated in the IM dimension, most of the coeluting positional were characterized by too little percent difference in CCS to be distinguished with the resolving power achieved in this study. According to the model developed by the group of McLean et al., baseline separation of ions that differ in CCS by less than 0.6% would require an ion mobility resolving power greater than 350, which is at least three times that achieved on our TIMS instrument (with \( V = 40 \text{ V/s} \)). Such resolving powers could potentially be achieved on ultra high resolving power instruments, such as the cyclic SLIM TWIMS platforms. Alternatively, ions having ACCS of 2% or more would only require resolving power below 100, meaning that the separation of the coeluting POP isobars in this study could be achieved on most modern IM instruments.

Conclusions:
This work demonstrated the potential of ion mobility as an additional dimension of separation to deal with the challenging coelutions of isomeric and isobaric species. While the coeluting isobars were structurally different enough to be baseline separated in the IM dimension, most of the coeluting positional were characterized by too little percent difference in CCS to be distinguished with the resolving power achieved in this study. According to the model developed by the group of McLean et al., baseline separation of ions that differ in CCS by less than 0.6% would require an ion mobility resolving power greater than 350, which is at least three times that achieved on our TIMS instrument (with \( V = 40 \text{ V/s} \)). Such resolving powers could potentially be achieved on ultra high resolving power instruments, such as the cyclic SLIM TWIMS platforms. Alternatively, ions having ACCS of 2% or more would only require resolving power below 100, meaning that the separation of the coeluting POP isobars in this study could be achieved on most modern IM instruments.

Acknowledgments:
This publication was supported by the French Community of Belgium through the funding of a FRIA grant (FC 47331). The authors would also like to thank the Belgian Federal Public Service of Health, Food Chain Safety and Environment through the contract RT22/06 TEQFOOD.

References:
MON-AM-D2  Assessing the performance of high resolution ion mobility-mass spectrometry for the separation of GC coeluting isomeric and isobaric halogenated persistent organic pollutants


**Overview of PFAS Testing Methods, Tips & Tricks**

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**Introduction:**
PFAS testing can be challenging due to their ubiquitous presence in our environment including the laboratory where the analysis is performed. Many published methods and articles address various workflows from direct injection to solid phase extraction and QuPPe from sample preparation viewpoint and from LC-MS/MS to GC-MS/MS from instrument platform viewpoint. This presentation will be an overview and guidelines when testing PFAS comparing currently available techniques and those under development along with some tips and tricks to pay attention. Most of the data are based on water testing ranging from ultrashort chain to mid and long chain PFAS compounds.

**Materials and Methods:**
There are mainly two approaches in PFAS analysis when it comes to aqueous samples: solid phase extraction and direct injection. Compilation of various methods will be reviewed in terms of sample preparation, sample volume, LC conditions and tips and tricks will be presented.

**Results:**
Precision and accuracy data for select PFAS compounds are shown below in the table with satisfactory results for water samples.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>%RSD</th>
<th>Mean Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perfluorobutanesulfonic acid (PFBS)</td>
<td>11.9%</td>
<td>91.1%</td>
</tr>
<tr>
<td>Perfluorohexanoic acid (PFHxA)</td>
<td>7.96%</td>
<td>99.4%</td>
</tr>
<tr>
<td>Hexafluoropropylene oxide dimer acid (HFPO-DA)</td>
<td>6.34%</td>
<td>94.4%</td>
</tr>
<tr>
<td>Perfluoroheptanoic acid (PFHpA)</td>
<td>4.19%</td>
<td>92.7%</td>
</tr>
<tr>
<td>Perfluorohexanesulfonic acid (PFHxS)</td>
<td>11.9%</td>
<td>89.4%</td>
</tr>
<tr>
<td>4,8-Dioxa-3H-perfluorononanoic acid (ADONA)</td>
<td>5.18%</td>
<td>96.6%</td>
</tr>
<tr>
<td>Perfluorooctanoic acid (PFOA)</td>
<td>5.21%</td>
<td>91.6%</td>
</tr>
<tr>
<td>Perfluorononanoic acid (PFNA)</td>
<td>6.79%</td>
<td>97.2%</td>
</tr>
<tr>
<td>Perfluorooctanesulfonic acid (PFOS)</td>
<td>6.78%</td>
<td>87.8%</td>
</tr>
<tr>
<td>9-Chlorohexadecafluoro-3-oxanone-1-sulfonic acid (9Cl-PF3ONS)</td>
<td>8.59%</td>
<td>85.1%</td>
</tr>
<tr>
<td>Perfluorodecanoic acid (PFDA)</td>
<td>6.96%</td>
<td>93.6%</td>
</tr>
<tr>
<td>N-methyl perfluorooctanesulfonamidoacetic acid (N-MeFOSAA)</td>
<td>10.1%</td>
<td>82.8%</td>
</tr>
<tr>
<td>N-ethyl perfluorooctanesulfonamidoacetic acid (N-EtFOSAA)</td>
<td>16.5%</td>
<td>106%</td>
</tr>
<tr>
<td>Perfluoroundecanoic acid (PFUnA)</td>
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<td>97.5%</td>
</tr>
<tr>
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<tr>
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<tr>
<td>Perfluorotridecanoic acid (PFTrDA)</td>
<td>12.7%</td>
<td>89.1%</td>
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<tr>
<td>Perfluorotetradecanoic acid (PFTA)</td>
<td>8.90%</td>
<td>89.7%</td>
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**Discussion and Conclusion:**
PFAS testing is evolving from water sample to food packaging and even to air samples. This presentation is to give an overview of various testing methods from differences, benefits, and disadvantages among them. Practical lab setting for PFAS testing, tips and tricks for better quality data are topics for discussion.

**Acknowledgments:**
US Environmental Protection Agency, ASTM
MON-AM-D4 Synthesis, purification, and structural characterization of linear isomers of native and deuterium-labelled perfluorooctane sulfonamido derivatives

Ana R. L. Araujo1,2*, Anton Pavlov2, Timo Hamers1, Marja Lamoree1, Sicco Brandsma1, Jon Eigil Johansen2, Huiling Liu2

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Introduction: Perfluoroalkane sulfonamido derivatives, including perfluoroalkane sulfoamides, sulfonamidoethanols, sulfamidoethyl acrylates and methacrylates, are important building blocks for a broad range of fluorotelomer products. Similar to perfluorooctanesulfonate (PFOS), perfluoroalkane sulfoamido derivatives have also been used in a variety of industrial, commercial and consumer products, and they have been the subject of interest because of their presence as global pollutants [1]. Due to the manufacturing process, technical PFOS derivatives, for example the sulfoamides, are not pure compounds but complex mixtures of linear and branched isomers, and other compounds including homologues. Questions have been raised as to whether the linear and the branched isomers behave differently in the environment. This work aims to synthesize, purify, and analyse pure linear isomers of perfluorooctane sulfoamido derivatives; and to create reference standards for this class of per-and polyfluoroalkyl substances (PFAS) compounds for environmental and toxicological studies.

Materials and Methods: Perfluorooctane sulfonamido derivatives and the deuterated analogues were synthesized by using perfluorooctane sulfonyl fluoride as the starting material. The commercial perfluorooctane sulfonyl fluoride was first reacted with benzylamine to give the solid sulfonamide as a key intermediate which is an isomer mixture containing about 75%-80% of linear isomer. The resulting mixture of isomers was submitted to recrystallization until we obtained the linear isomer. Isomer profile analysis was done by UHPLC using different columns (C18, biphenyl and PFAS columns) and GC-MS. The purified n-isomer was converted to other sulfonamides after further alkylation of the nitrogen and removal of the benzyl group by catalytic hydrogenation, followed by a reaction with bromoethanol to synthesize sulfonamidoethanols. The last step of the synthetic route was the esterification of the amino alcohol sulfonamide with methacryloyl or acryloyl chloride. When needed, the compounds were purified by Dry Column Vacuum Chromatography (DCVC) [2], and 1H NMR, 19F NMR, and GC-MS were used for purity assessment.

Results: For the synthesis of this group of compounds, using perfluorooctane sulfonyl fluoride as starting material built a large-scale and efficient synthesis for the different synthetic routes. The linear isomer of the benzylamine sulfonamide was purified to > 99% purity. The recrystallization method showed to be highly efficient, and the final methacrylate and acrylate derivatives were easily purified using DCVC column technique. All products were obtained with good yield and high purity (>98%), based on GC-MS analysis.

Discussion and Conclusion: The 4 native and 4 labeled standards of perfluorooctane sulfonamido derivatives were synthesized successfully as linear isomers. The study showed that recrystallization of the key intermediate is an efficient method to remove possible isomers and a DCVC can be used as a great tool to purify the products. All compounds were characterized by 1H NMR, 19F NMR, and GC-MS and showed high purity to be used as new reference materials for both environmental and toxicological studies.

Acknowledgments: This project has received funding from the European Union’s Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement REVAMP project No 956374.

References:
Progress in Methods for POPs Analysis

W.Tirler & G.Eppe

MON-AM-D5 Analysis of Ultrashort-Chain and Short-Chain (C1 to C4) Per- and Polyfluorinated Substances in Potable and Non-Potable Waters

Mike Chang1, Jamie York1, Shun-Hsin Liang1, Justin Steimling1

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**Introduction:** Perfluoroalkyl substances are a group of man-made chemicals widely used in industrial applications and consumer products. Their widespread usage and resistance to degradation has resulted in PFAS being a ubiquitous environmental contaminant and the potential health effects is of growing concern. While many of the long-chain PFAS have been recognized as harmful, alternative compounds have emerged in their place. Short-chain PFAS compounds are considered to be less bioaccumulative and toxic than long-chain, but their widespread use has resulted in their increased environmental accumulation. In this work, the analysis of ultrashort-chain and short-chain (C1 to C4) per- and polyfluorinated substances is outlined and applied to potable and non-potable waters by LC-MS/MS.

**Materials and Methods:** Reverse osmosis water was collected from the facility of Restek Corp. (Bellefonte, PA). Wastewater samples were gifts from GDIT (Falls Church, VA). Bottled waters were obtained from local grocery stores. Norm-Ject syringe (10 mL Luer Lock Tip) and syringe filter (30mm, 0.45 µm Nylon) were obtained from Restek Corp. (Bellefonte, PA) and used for the preparation of standard and sample solution. The analysis was performed on the Waters Acquity UPLC system coupled to a Xevo TQ-S triple quadrupole mass spectrometer. Compound tuning was conducted using negative mode of electrospray ionization to determine precursor and product ions. A Raptor Polar X column (50 x 2.1mm, 2.7 µm) from Restek Corp. (Bellefonte, PA) was used for chromatographic analysis of seven C1 – C4 PFAS analytes. The aqueous mobile phase (Mobile Phase A) was a mixture of 10 mM ammonium formate and 0.1% formic acid in water and the organic mobile phase (Mobile Phase B) was 0.1% formic acid in 95:5 acetonitrile:isopropanol. Separation was performed with the isocratic elution under 85% B for 7 minutes of each injection of 10 µL of standard and sample solutions. The flow rate was 0.3 mL/minute, and the column temperature was controlled at 40° C.

**Results:** An LC method was established with the aim to obtain the best MS detection sensitivity and yet could mitigate the matrix interference. With linear regression (1/x weighted), all analytes showed acceptable linealities with r² >0.995 and deviations <20% at the range of 2.5 – 800 ppt for C1 – C4 PFSAs, 5.0 – 800 ppt for PFBA and PFPrS, and 20 – 800 ppt for TFA. The tap water, spring bottled water, and potable water (POTW) water were fortified at 25, 50, and 175 ppt for all analytes. Three batches of analyses were performed on different days for a total of nine repetitions at each fortified level. There was differential amount of TFA in all 3 water samples. The tap water had incurred TFMS as well. In addition to TFA and TFMS, the POTW water also contained PFBS and PFPrA. These incurred concentrations were subtracted from the calculated concentrations of fortified samples to determine the recovery. Due to a much higher TFA concentration in the POTW water, the determination was performed on a 5-fold diluted (in RO water) POTW water for the accuracy and precision analysis of TFA. There was no data to be collected for TFA at 25 ppt fortified concentration as it was unable to obtain accurate quantification by concentration subtraction of incurred TFA. All analytes had recovery values of 86.6 - 107% across three fortification levels among three different types of waters. Satisfactory method precision was demonstrated with %RSD values within 1.62 to 10.7%. This direct injection workflow was applied to the determination of C1 to C4 PFAS in a variety of tap waters, bottled waters, natural spring water, well water, and wastewaters from different sources. Three preparations of blank and fortified (50 ppt) samples were injected for the analysis. It was shown that the averaged recoveries of fortified QC samples were all within 75 to 120%. This demonstrated that the established method was suitable for accurate measurement of C1 to C4 PFAS in both potable and non-potable waters. The data indicated that TFA was ubiquitously present in tap waters at the range from ~120 to 500 ppt. TFMS was present and quantifiable in most tap waters and PFPrA was detectable in several tap waters. The tested spring bottled waters contained TFA as well. It was clean of C1 to C4 PFAS for an RO purified bottled water and an RO filtrated tap water. The well water tested had a relatively higher amount of TFMS. The wastewaters originated from POTW, hospital, metal finisher, and chemical manufacturer all had higher levels of TFA and PFPrA. The wastewater effluent collected from a chemical manufacturer had significantly elevated levels TFA, PFPrA, and PFBA contamination.

**Discussion and Conclusion:** A direct injection workflow was established to provide a unique solution for the determination of ultrashort-chain and short-chain PFAS in various water matrices. The reported method was rugged, accurate, and precise implementing a fast 7-minute chromatographic analysis. Most importantly, this solution can offer a great tool for the monitoring of these emergent PFAS in environmental water system and assist in generating a guideline for future regulatory references. Other application areas such as food contact materials will be a great area for exploring the possibility of this technology.

**Acknowledgments:**
US Environmental Protection Agency, ASTM
Introduction: Smoking is a traditional method of food processing that remains widely used today. Due to the unique texture and flavor of smoked meat, many families in southern China continue to smoke pork at home using traditional methods every winter. However, incomplete combustion of fuels during the smoking process can also lead to the formation of various chemical contaminants, such as PCDD/Fs. Therefore, the objectives of this study were to investigate the contamination characteristics of PCDD/Fs in pork smoked at home in China, and assess the potential health risks associated with consuming such food.

Materials and Methods: A total of 30 samples of traditional smoked pork were collected from fifteen families from eight cities in five provinces (Sichuan, Yunnan, Guizhou, Guangxi, Hunan) in southwestern China. Each piece of smoked pork was divided into two parts: the surface (0.5 cm from the surface) and the inner (1.5–2 cm away from the surface). In addition, 11 samples of fresh raw pork from the same areas were also collected. The smoked pork collected was produced in a similar way. The pork was first salted and then hung above the fuel for smoking. The samples were extracted by an accelerated solvent extractor. Then, the extracts were purified by chromatographic column method. Finally, the concentrations of 17 PCDD/Fs in samples were determined by GC-MS/MS. The detail of column purification procedure and instrumental method can also be found in our previous study. The recovery of 13C12-labeled PCDD/Fs in samples was in the ranges of 64–135%.

Results: The TEQs of PCDD/Fs in traditional smoked pork samples ranged from 3.96 to 162 fg TEQ/g wet weight (29.7 to 501 fg TEQ/g fat weight), which did not exceed the maximum limit set by EU for PCDD/Fs in pork (1 pg/g fat). The health risk assessment was based on the data detected in this study and the dietary survey of the local residents at the sampling sites. The average daily exposure doses (ADDs) of PCDD/Fs in traditional smoked pork samples ranged from 0.666 to 68.9 fg TEQ/kg d (average: 17.4 fg TEQ/kg d), lower than the tolerable daily intake proposed by WHO (1–4 pg TEQ/kg d). The cancer risk (CR) of PCDD/Fs in traditional smoked pork samples ranged from 1.04x10^{-3} to 1.07x10^{-5}.

Discussion and Conclusion: To the best of our knowledge, this is first study to investigate the levels, enrichment characteristics and dietary intake risk of PCDD/Fs in Chinese traditional smoked pork. The total concentration of PCDD/Fs (PCDD/Fs) in traditional smoked pork samples (average: 2.15 pg/g fw) was significantly higher than those in raw pork samples (average: 0.742 pg/g fw). Furthermore, the detection rates and concentrations of seventeen PCDD/F congeners were increased in smoked pork samples compared to the corresponding raw pork samples. The concentrations of 2378-TCDF, 12378-PeCDF, 23478-PeCDF, 123678-HpCDF, OCDF, 1234678-HpCDF and OCDD were significantly higher in smoked pork than in raw pork (p < 0.05), indicating that these seven congeners may be the primary PCDD/Fs enriched in pork during traditional smoking. These congeners were called the enriched congeners in this study. The concentrations of seven enriched PCDD/F congeners between the inner and surface parts of smoked pork were further compared. It was found that the concentrations of OCDF and 1234678-HpCDF have no statistical difference between the two groups (p > 0.05), suggesting that they may be easier to transfer from the surface to the inner part of smoked pork. These results suggested that the traditional smoking way may exacerbate the contamination of PCDD/Fs in pork. Although the CR of all samples to the local residents did not exceed the unacceptable threshold (10^{-4}), 60.0% of them posed a potential carcinogenic risk (>10^{-4}). It is worth noting that only PCDD/Fs were considered in this study. PCDD/Fs are not only found in smoked pork, but also in other common foods. According to the fifth China total diet study (CTDS), the estimated dietary intakes of PCDD/Fs for adult males are 0.12, 0.55 and 0.91 pg TEQ/kg/day in Sichuan, Guangxi and Hunan province, respectively. Although the ADDs of PCDD/Fs from traditional smoked pork was lower than the CTDS result, the proportion of smoked pork to CTDS of Sichuan, Guangxi and Hunan provinces reached 32.6%, 1.46%, and 4.59%, respectively. Especially in Sichuan, the ADDs of PCDD/Fs in smoked pork has exceeded the contribution of various other foods, such as aquatic foods, egg and egg products, milk and dairy products, and has become a major contributor of CTDS.

Therefore, the level of PCDD/Fs present in smoked pork may be an important factor contributing to health hazards. The results of this study can provide valuable data for comprehensively assessing the risk of exposure dioxins among residents in areas where traditional smoked pork is commonly consumed in China.

References:
**MON-AM-E2**  
**Dioxin-like Compounds by DR CALUX in Free-range Chicken Eggs**

Nikola Jelinek¹*, Peter Behnisch², Jindrich Petrik¹,², Lee Bell³, Miroslava Jopkova¹, Lenka Petrikikova Maskova¹, Dmitriy Kalmykov⁴, Emiel Felzel⁵, Punyathorn Jeungsmarn⁵, Thitikorn Boontongmai⁵, Akarapon Teebthaisong⁵

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² BioDetection Systems B.V., 1098 XH Amsterdam, The Netherlands  
³ International Pollutants Elimination Network (IPEN), Gothenburg, Sweden  
⁴ Karaganda regional ecological museum (EcoMuseum), Karaganda, Kazakhstan  
⁵ Ecological Alert and Recovery Thailand (EARTH), Nonthaburi, Thailand

**Introduction:**
There is a range of studies on polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) and polychlorinated biphenyls (PCBs) in eggs. Free-range poultry eggs have been found to be sensitive indicators of PCDD/F and PCB contamination in soils and are an important exposure pathway from soil pollution to humans¹. Eggs from contaminated areas can readily lead to exposures which exceed thresholds for the protection of human health²⁻⁵. Chickens and/or ducks and their eggs might therefore be considered ideal "active samplers" and indicator species for persistent organic pollutants (POPs) contaminated sites.

The International Pollutants Elimination Network (IPEN) in cooperation with local civil society organizations (CSOs) have conducted the first global study about dioxin, PCBs and HCB contamination of free-range eggs from sites in 17 countries worldwide suspected to be polluted by POPs in 2005⁶. Almost twenty years work by IPEN and Arnika in this field, combined with data from other studies on this topic, were summarized in a global review of PCDD/Fs and dl PCBs in free-range chicken eggs⁷. Many samples collected in cooperation with more than 50 national or local CSOs were also analyzed by Chemical Activated Luciferase gene eXpression (CALUX) bioassay analyses for dioxin activity (DR CALUX). These analyses are presented in this report and compared with other publicly available studies using DR CALUX for evaluation of dioxin-like compounds in poultry eggs. A summary of the entire range of data has now been prepared to supplement our previous report.

Some data about dioxin-activity measured in broader environmental samples taken at selected POPs hot spots are available in previous reports from Armenia⁸, Kazakhstan⁹, Thailand¹⁰, Indonesia¹¹, Ghana, Cameroon¹², Bosnia and Herzegovina, Montenegro and Serbia¹³, Lithuania, Spain and Czechia¹⁴. Quite broad research on POPs in eggs was also conducted in Arusha, Tanzania¹⁵. Recently DR CALUX was used in food and/or egg monitoring reports in Netherlands¹⁶, Germany¹⁷ or Kuwait¹⁸. In addition, all of these reports also included DR CALUX results for free-range eggs used in this study.

**Materials and Methods:**
Dioxin activity was analyzed by DR CALUX bioassay in 64 pooled free-range poultry egg samples collected from 36 hot spots, 3 remote localities and four reference samples from supermarkets and in 18 countries since 2010 until 2023. Obtained results from hot spots and remote areas were compared with analyses of 4 reference samples of eggs from supermarkets. The majority of the analytical results presented in this study come from larger reports released in 2011 - 2018, where more information about the sites and sampling can be found⁹⁻¹²,¹⁴. Normally between 2 to 6 eggs were pooled for analysis of one flock.

Pooled egg samples were analyzed for PCDD/Fs as well as for dioxin-like polychlorinated biphenyls (dl-PCBs) using the DR CALUX method. The samples were sent to a Dutch ISO 17025 certified laboratory BDS performing the cell-based screening analysis DR CALUX according to the European Standard EC/644/2017. The procedure for the BDS DR CALUX bioassay has been described in detail by Besselink, et al.¹⁸.

Briefly, rat liver H4IIE cells stably transfected with an AhR-controlled luciferase reporter gene construct were cultured in an α-MEM culture medium supplemented with 10% (v/v) FCS under standard conditions (37°C, 5% CO₂, 100% humidity). Cells were exposed in triplicate on 96-well microtiter plates containing the standard 2,3,7,8-TCDD calibration range, a reference egg sample (for the bioassay apparent recovery), a procedure blank, a DMSO blank and the sample extracts in DMSO. Following a 24-hour incubation period, cells were lysed. A luciferin-containing solution was added, and the luminescence was measured by using a luminometer (Mithras, Berthold). The DR CALUX bioassay method has been shown to be a cost-efficient, semi-quantitative, effect-based toxicity screening analysis for all kinds of stable dioxin-like compounds (PCDD/Fs, dl-PCBs, PBDD/Fs, PBBs, and chlorinated and brominated polycyclic aromatic hydrocarbons, N-dioxins).
### Results:
Levels of PCDD/Fs + dl PCBs measured by DR CALUX in 64 pooled egg samples are summarized in Table 1. The highest levels exceeding 20 pg BEQ/g fat in free-range poultry egg samples including those from reviewed literature are presented in Figure 1.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Country</th>
<th>Locality</th>
<th>Year</th>
<th>DR CALUX (PCDD/Fs + dl PCBs)</th>
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**10:30 - 11:50**

**Levels and Trends (Foods and Feeds)**

*M. Rose & H. Vanderperren*

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<td>Ghana - Kumasi - hospital 2018 5.2 WI</td>
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<td>Kenya - Nanyuki dumpsite 2021 4.9 DU</td>
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<td>165</td>
<td>UK - Bishop’s Cleeve 2011 1.8 WI</td>
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<td>Montenegro - Plužine - Orah 2015 0.98 Rem</td>
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<td>153</td>
<td>Armenia - Mushavan 2010 &lt;LOQ (0.79) OCPs</td>
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<td>19</td>
<td>Indonesia - Jakarta - supermarket 2019 &lt;LOQ (0.6) Ref</td>
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Measured levels in free-range eggs ranged from a background level below LOQ (0.79) pg BEQs/g fat in sample from Mushavan, Armenia up to high level of 840 pg BEQs/g fat of a pooled eggs sample from Agbogbloshie, e-waste scr in Ghana. Levels in four reference samples were in the range of below LOQ (0.79) to 1.2 pg BEQs/g fat.

**Discussion:**

Only 13 out of 64 samples were below the level of 5 pg BEQs/g, the EU health protective standard for eggs\(^1\). The rest (51) of 64 samples were suspected to be contaminated by dioxin and dioxin-like compounds above the EU food standard. This should be expected as most localities were selected due to the activity generating PCDD/Fs and dioxin-like compounds.

Figure 1 compares highest levels of PCDD/F\(+\) dl PCB measured by DR CALUX in free-range chicken eggs from various locations including those from scientific literature\(^{1,11,12,19,20}\). When we compared them according to most likely sources of contamination, e-waste sites seem to be the most critical locations with 7 out of 10 highest levels measured in egg samples from e-waste sites in Africa (Ghana and Kenya) and South-east Asia (Thailand, Indonesia and Philippines). High levels of dioxin activity was also observed in eggs from the vicinity of places affected by use of Agent Orange, a defoliant contaminated with dioxins\(^{19}\). In eggs from another site with buried DDT and other OCPs in Nubarashen, Armenia\(^2\) there was also relatively high level of 37 pg BEQ/g fat.
Levels above 20 pg BEQ/g fat were also measured at sites contaminated as a result of improper handling of waste incineration ashes (Bishop’s Cleeve, UK and Accra – Hospital, Ghana)\textsuperscript{12,23} and/or from the vicinity of waste incinerators (Aguado, Philippines, Lisbon, Portugal, Wuhan, China and Plzen, Czechia)\textsuperscript{11,14,20,24}. There were 28 and 20 pg of PCDD/Fs + dl PCBs measured by DR CALUX in BEQ/g fat in samples from the vicinity of waste incinerators in Lisbon\textsuperscript{20} and Plzen\textsuperscript{11} respectively. Samples potentially influenced by waste incineration have shown the levels in the range of 1.8 to 66 pg BEQ/g fat. Eggs from sites contaminated with either OCPs or PCBs and by chlorine chemical industry had levels from below LOQ (0.6) to 37 pg BEQ/g fat.

Some of the very high observed levels 101, 37, and 28 pg BEQs/g fat respectively were from Balkhash (Kazakhstan), Beihai (China), and Temirtau (Kazakhstan). All these localities are influenced by metallurgical industry. The map at Figure 2 shows samples and DR CALUX levels of dioxin activity measured in pooled free-range eggs from the vicinity of metallurgical complex in Beihai, China. These findings confirm previous study findings that “large metal industries can pollute areas up to 20 km”\textsuperscript{4,25}. Levels in eggs from the vicinity of metallurgical plants varied between 8.9 and 101 pg BEQ/g fat from Beihai, China and Balkhash, Kazakhstan respectively. Metal smelters are significant sources of contamination with PCDD/Fs, and dl PCBs in particular.

There are 7 samples from e-waste sites among ten highest levels recorded clearly indicating that open burning or incineration of e-waste and plastic treated with brominated compounds are significant sources of PCDD/Fs, dl PCBs and PBDD/Fs. E-waste sites are among those with highest measured levels of PBDD/Fs in free-range eggs\textsuperscript{26}. We can estimate that PBDD/Fs significantly contributes to DR CALUX levels in eggs from e-waste sites\textsuperscript{9,27}.

Conclusions:
We found DR CALUX bioanalysis to be a very useful alternative to chemical GC/HRMS based analysis especially for projects with limited funds or opportunity to order chemical analyses of dioxins. It also helped us to decide about follow-up steps and to select samples for analyses for PBDD/Fs. E-waste, metal industry, and waste incineration sites respectively are location types where the highest levels of dioxin activity with DR CALUX were measured in free-range eggs. These locations include sites contaminated as a result of improper handling of waste incineration ashes and highlight the need for more strict limits for regulation of PCDD/Fs and dl PCBs in wastes. It is recommended to add DR CALUX testing in eggs surrounding such sites.

Acknowledgments:
The study was financially supported by EU Aid grants for work in Armenia (2010-2011), Kazakhstan (2012-2015), China (2013-2015), Thailand (2015-2019), and Indonesia (2020-2023); Government of Sweden the Global Greengrants Fund, and the Sigrid Rausing Trust for work on the whole study, and Ministry of Foreign Affairs of the Czech Republic for work in Thailand, Armenia and Indonesia.
References:


MON-AM-E2  Dioxin-like Compounds by DR CALUX in Free-range Chicken Eggs

Elevated levels of dioxins, PCBs and PFASs in wild cows grazing in flood plains of large rivers in the Netherlands

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**Introduction:** To manage the vegetation in flood plains of Dutch rivers, specific breeds of wild cows and horses are used, which forage year round in these areas, unless an incidental periods of flooding does not allow this. For herd management reasons, occasionally, animals are removed from the herds and the meat is sold as wilderness meat. Since soil in these areas may contain elevated levels of dioxins and PCBs, research was started to investigate the contaminant levels in fat, meat, livers and kidneys, as well as soil and grass from flood plains. Also levels of PFASs were investigated, as well as heavy metals (data not presented).

**Materials and Methods:** Samples of perirenal fat, liver, meat and kidney from animals were obtained at the slaughterhouse and provided by the owners of the animals, being a number of foundations. Samples were analysed by routine methods for dioxins, PCBs and PFASs applied at WFSR, which is the National Reference Laboratory in the Netherlands.

**Results:** Fat and livers of most animals were shown to contain levels of dioxins and PCBs (dioxin- and non-dioxin-like) exceeding the current EU maximum levels (MLs) for bovines. Lipid based levels in fatty meat were similar as in perirenal fat but were lower in lean meat (<1% fat). Lipid based levels in liver were higher than in perirenal fat, due to sequestration. PFAS levels and in particular those of PFOS were elevated in livers and meat, those for PFOS being several-fold higher than the new EU ML. There was a tendency for decreased contaminant levels in animals slaughtered later in the year.

Soil levels of dioxins and PCBs were shown to be higher compared to levels in pastures on the other side of the dikes. Levels in vegetation were rather low, except after a period of flooding when soil was attached to the surface of the grass. Soil appears to be the main source for the contamination of the animals, with a relatively high intake during the winter period when grass is short and especially after a flooding episode. This also seems to apply for PFASs, although lower LOQs are required to exclude a high contribution of grass.

Animals that were transferred to a stable and provided with clean feed, showed a sharp decrease in the levels of dioxins, PCBs but also PFASs. Levels in these animals were also monitored via the blood.

Levels in the estuary of the Rhine were higher than for the Meuse, and also showed a different congener pattern. Especially the much higher contribution of TCDD to the TEQ levels was noticed, pointing at a specific, trichlorophenol-related, source along the Rhine river. The relative contribution of dl-PCBs to the TEQ levels was higher in animal tissues than in soil, suggesting a difference in kinetics.

**Discussion and Conclusions:** The results show that levels of dioxins, PCBs but also PFASs in tissues of cows grazing in flood plains of large rivers in the Netherlands are relatively high compared to other cattle and as such exceed the MLs for dioxins and PCBs but also the new MLs for PFASs. Although based on just a few animals, levels seem to decrease during the season, due to lower intake of soil during the summer, growth of the animals, increase in fat content and possibly also excretion. This was supported by the data from animals transferred to a stable with clean feed. Therefore, slaughtering at the end of the season is an important measure, but also avoiding the high intake of soil after a flooding episode. For dioxins and PCBs, the fatty tissues are the most important source of elevated human exposure.

**Acknowledgments:** This work was sponsored by the Dutch ministries of Public Health and Agriculture. In addition the authors would like to thank the support of the Dutch Food and Consumer Products Authority (NVWA) and the Foundations that provided the samples of the animals, as well as the many technicians at WFSR that performed the analyses.

**References:**
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Combining Quantification of Suckling Cow Exposure and PBTK Modelling for Risk Assessment of Beef Meat Contamination with dioxin-like PCBs

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Introduction: Swiss and German surveys of polychlorinated biphenyls (PCBs) contamination of meat from suckling cow herds have revealed occasional exceedances of the regulatory maximum level (ML, EU 1259/2011 regulation). Some of these contaminations could be attributed to diffuse sources of PCBs coming from soil ingestion, which is presumably higher in grass-based feeding systems. A proper risk assessment of exceeding ML in meat relies on i) estimating cow oral exposure to PCBs (i.e., feed and soil intakes) and ii) quantifying the transfer of PCBs from oral dosing to cow meat. The aims of the present work were to i) aggregate monitoring data from different databases for estimating PCB exposure and ii) quantify the corresponding PCB transfer into beef cow using a physiologically-based toxicokinetic (PBTK) model.

Materials and Methods: The quantification of the exposure to the 12 dioxin-like PCBs (dl-PCBs) of Swiss suckling cow herds have been estimated by collecting and merging 18 databases from scientific publications, Swiss federal and cantonal administrations, and the Swiss Mother Cow association. Combined exposure through feed (forages and concentrates) and soil ingestions was considered. Typical feeding systems for suckling cow husbandry in Switzerland were studied (mountains, hilly and lowlands areas, and farms using concentrate feeds, during winter and summer seasons). Three different levels of soil intake were assumed, covering the range measured in beef heifers at pasture: 1%, 3%, and 9% of total dry matter (DM) intake. The distributions of the concentrations in individual dl-PCB congeners in each feedstuffs entering suckling cow rations [fresh grass, hay, grass silage, corn silage, concentrates and "other"; n=132], as well as in Swiss rural and agricultural soils (n=26) were determined. Median, third quartile (Q3) and ninth decile (D9) exposure scenarios were further computed for each feeding systems and soil ingestion levels. The corresponding total diet dl-PCB concentrations were further used as input parameters into a PBTK model describing the fate of lipophilic contaminant in lactating cow, formerly adjusted for dl-PCBs using beef cow PCB toxicokinetic data as a calibration dataset.

Results: The highest median dl-PCB level was observed in soil (0.38 ng TEQ/kg DM) followed by grass silage (0.13), hay (0.12), pasture (0.12), and other feedstuffs (in average 0.05). All the feeding systems (i.e., mountains, hilly or lowlands areas) comprised more than 85% (DM basis) of grass-based forages (mostly hay and grass silage during winter and pasture during summer). This led in nearly similar diet dl-PCB concentrations, across feeding systems and winter or summer seasons, which depended mainly on soil intake level and Median (in average 0.12, 0.12 and 0.14 ng TEQ/kg DM, for 1, 3 and 9% of soil), Q3 (0.17, 0.18 and 0.20) or D9 (0.24, 0.27 and 0.35) scenarios. None reached the regulatory feed action threshold (AT) set at 0.40 ng dl-PCBs TEQ kg-1 (277/2012 EU regulation). Nonetheless, according to the suckling cow PBTK model run over five consecutive lactations, dl-PCB concentration in adipose tissues of the D9 scenarios might achieved up to 2.7, 3.0 and 3.9 pg TEQ g-1 lipids for 1, 3 and 9% soil intake, respectively. Those levels are however overpassed for Q3 scenarios (maximum of 2.0-2.3 pg TEQ g-1 lipids).

Discussion and Conclusion: The present study highlights that even with diet dl-PCB levels lower than feed AT, there is a risk of exceeding the AT in meat of suckling beef cows for scenarios where feed and soil are contaminated above the median level (scenarios Q3 and D9). Investigations also confirm that when punctual sources (e.g., PCB-loaded barn materials and equipment, accidentally contaminated feedstuffs) are excluded from calculations, a major route of exposure to dl-PCBs is through soil ingestion. Additional monitoring data regarding PCB contamination levels in feeds and soils and their temporal trends would be needed to strength such quantitative risk assessment. This requires expanding and merging more federal, cantonal, and industrial monitoring plans and surveys. Ultimately, the methodological framework, based on combining disperse databases and PBTK modeling, will improve the decision-making process of feed and food safety risk assessors, and may further be extend to other contaminants (ndl-PCBs, PCDD/ Fs, emerging POPs...) and farm animals (dairy ruminants, poultry, swine...). This would help advising farmers in order to avoid critical scenarios and prevent livestock contamination incidents.

Acknowledgments: This study was co-funded by the Federal Office for the Environment (FOEN, project MeatPOP n°17.0082.PJ) and the Swiss Mother Cow association.

References:
**MON-PM1-A1 Distribution of per- and polyfluoroalkyl substances in fish organs from Michigan**

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**Introduction:** In response to the growing PFAS concern, the Michigan Ecology Center partnered with other organizations and community anglers to collect and analyze filet, gut (stomach plus intestines), liver, and eggs in multiple fish species from popular fishing areas for PFAS. The direct oxidizable precursor assay was shown to be useful to investigate the presence of precursors that can’t be detected using targeted analyses. This study compared the results to current fish consumption guidance and identified potential hot spots and sources of contamination, which can inform interventions to reduce PFAS exposure in the future.

**Materials and Methods:** The study composited over 100 different fish from six different species to measure PFAS in intestines, stomach, eggs, filet, and liver using a targeted suite of 66 PFASs. A subset of samples was also analyzed using total direct oxidizable precursor assay (dTOP, n=6) and total extractable organic fluorine (EOF, n=22). All analytical techniques followed previously reported protocols.

**Results:** Spearman correlation showed a positive relationship ($P<0.0005$) between the $\sum$PFAS and EOF, indicating a significant overlap of targeted and EOF compounds. The median PFOS level in fish filet, gut, egg, and liver was 4.5, 16, 23, and 38 ng/g wet weight (w.w.), respectively. Of eight branched-chain PFAS, only three were common (i.e., P37DMOA, P3MHpS, and P6MHpS) in the fish organ samples. The dTOP assay showed the highest percentage increase in targeted PFAS in the Catfish fillet (>500%) while the smallest percentage increase was observed in the Catfish liver (32%) compared to the original levels. Catfish fillet showed an increase of 1.3 nMole F g$^{-1}$ dry weight (d.w.), whereas Catfish liver showed an increase of 8.1 nMole F g$^{-1}$ d.w. The median levels of $\sum$PFASs in filet from Huron and Rouge watersheds were 13 ± 16 and 6.3 ± 1.6 ng/g w.w., respectively.

**Discussion:** In this work, the levels of PFOA, PFOS, PFNA, PFHxS, and PFBS in all liver, egg, and intestine samples exceeded the limited consumption guidelines, while 9 of the 11 filet samples exceeded the guidelines and none exceeded the “do not eat” advisory. Furthermore, the PMF analysis showed that three PFAS sources were identified, with a factor dominated by PFOS explaining 73% of the data.

**Conclusions:** This study identifies potential sources of PFAS contamination in fish tissues and emphasizes the need for continued monitoring and investigation of the associated health risks. Results suggest that consuming PFAS-contaminated fish may present a potential health risk not only for humans but also for the broader ecosystem. High levels of PFAS in fish organs indicate greater risks to the ecosystem, underscoring the importance of ongoing monitoring of PFAS levels in aquatic organisms, particularly in areas with known contamination.

**Acknowledgments:** The study was only possible with the knowledge and fishing skills of the anglers, Friends of the Rouge, and Huron River Watershed Council. Although this work was sponsored by the Community Foundation of Southeast Michigan, it reflects the views of the authors and does not represent the policy endorsed by this organization.

**References:**


MON-PM1-A2  Experimental Study to determine a Transfer of PFAS in Beeswax and from Beeswax to honey

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Introduction: PFAS occur in many food matrices. Also in honey PFAS were found [1]. To date it is not clear how exactly PFAS enter honey. One possible explanation is, when bees collect PFAS-containing nectar, water and pollen [1]. The bees store honey in beeswax. The question rises, if PFAS from honey can enter beeswax. Also the other way round, beeswax itself might be a source of contamination for honey. Beeswax is reused by the beekeeper by melting and producing new central walls and start stripes. In this way contaminants could persist and potentially enter honey; this has been described for the veterinary drug class nitroimidazoles [2]. No data are available proving a transfer of PFAS in beeswax and vice versa. The aim of the study was to determine a transfer of PFAS into beeswax and from beeswax to honey dependent from the chain length.

Materials and Methods: In our study we used linear perfluorinated carboxylic acids (PFCA, chain-lengths of 4 to 18 C-atoms) and linear perfluorinated sulfonic acids (PFSA, chain-lengths of 4 to 8 C-atoms). To determine a potential transfer of PFAS from honey to beeswax, blank honey was spiked with 20 µg/kg PFAS, then filled into Falcon tubes coated with blank beeswax and incubated at 37 °C for up to 42 days to mimicking conditions in the beehive. In another experiment, spiked beeswax (500 µg/kg PFAS) was incubated at 65 °C for 24 h with water (pH 4). Finally, blank beeswax was incubated at 65 °C for 24 h with spiked water (20 µg/kg PFAS) at pH 2, 4 and 6 to determine the influence of pH on the transfer rate considering the pH range of honey. Extraction of PFAS from beeswax and water was done using a modified QuEChERS protocol with formic acid after addition of isotopically labeled internal standards and clean up via Envi-Carb SPE tubes. For analysis, an Agilent 1260 Infinity LC coupled to an Agilent 6500 Triple quadrupole tandem mass spectrometer (Agilent, Santa Clara, CA) interfaced with an electrospray ion source working in the negative ion mode was used.

Results: In our experiments we observed a transfer of several PFAS from spiked beeswax into water, dependent on the chain-length. While short chain PFAS almost completely entered the aqueous phase, C12-PFSA (PFDoS) nearly completely remained in the beeswax. The transfer of PFCAs from C4 to C18 reached a plateau from chain-length of 10 C-atoms. At the same time, with increasing chain-length, less PFAS were found in water. This behavior is based on the logKow being lower for short-chain PFAS. We also found an influence of the pH value. With increasing pH less short chain PFAS entered beeswax. Interestingly, this tendency reversed for PFCAs from a chain-length of 12 C-atoms. Finally, a transfer of PFAS from spiked honey into beeswax was observed. This transfer was very low and not observable for the longest chain PFCA of the study (PFODA, 18C).

Discussion and Conclusion: Using model conditions, a transfer of PFAS from water into beeswax and inversely is possible. Nevertheless, this simplified model gives limited information about the actual transfer behavior in the beehive, as our experiment with honey and beeswax showed. A lot of parameters might influence the transfer rate. Honeys for example not only differ in pH, but also contain different amounts of compounds such as sugars, pollen, proteins, amino acids and vitamins. In European honey PFAS were found in a range up to 0.9 µg/kg [1]. An option to be further investigated is the analysis of PFAS in beeswax as indicator for environmental pollution, given that some PFAS persist in beeswax.

References:
MON-PM1-A3  Ultra-short chain PFAS: Understanding Measurement, Occurrence and Fate in North America

Bharat Chandramouli, Million Woudneh, SGS

Introduction:
Per-and Polyfluoroalkyl Substances (PFAS) with chain lengths of 3 or fewer are referred to as ultra-short PFAS and there is a significant data gap on their occurrence, fate and toxicity information. Ultra-short PFAS of particular interest include trifluoroacetic acid (TFA), pentafluoropropionic acid (PFPrA) and corresponding sulfonic acids trifluoromethane sulfonic acid (PFMS), and the C2 and C3 perfluorinated sulfonic acids PFEtS and PFPrS. These acids have multiple sources into the environment with TFA arising from atmospheric degradation of hydrofluorocarbons (HFCs and HCFCs), the oxidation and chain shortening of fluorotelomer PFAS and more. PFPrA is also likely formed from the degradation of fluorotelomer PFAS. PFMS is used widely in organic synthesis and in lithium ion batteries. The limited information on TFA available in the literature suggests that background concentrations are steadily increasing. LC-MS/MS methods used for short and long-chain PFAS do not typically work for ultra-short PFAS necessitating new approaches. Our objective was to develop and validate a suitable method for ultrashorts and use it to understand occurrence in the environment.

Materials and Methods:
We developed and validated an isotope dilution/surrogate standard quantitation UPLC-MS/MS method for the measurement of 5 ultrashort PFAS TFA, PFPrA, PFMS, PFEtS and PFPrS in aqueous samples. Reporting limits for the method ranged from 1-20 ng/L. The analytical approach was designed to be fully compatible with the preparation protocols from EPA 1633 enabling measurement of the ultrashorts from the final preparation step of EPA 1633. Two different LC columns were tested, a reversed phase plus anion exchange column (Waters, Atlantis Premier BEH C18 AX) as well as an ion exchange and HILIC column (Restek, Raptor polar X). The mixed ion exchange and HILIC column was used to validate the method. Retention and elution of the analytes through sample extraction/cleanup was also investigated using Waters WAX SPE cartridges.

Results:
Quantitative retention and elution (>95%) of the analytes was achieved using the conditions described in the US EPA method 1633 coupled with the new LC-MS/MS method. In a reagent water spike /recovery experiment (n=5) recovery values of 76-106% and RSD values of 0.8-2.4 was achieved. Initial tests indicated suitability in surface water as well, and additional robustness data will be presented. This study will present results from a US study in wastewater that also included target PFAS information for the 40 PFAS in EPA 1633 and results from the total oxidizable precursor assay (TOP).

Discussion and Conclusions:
Method validation met all performance criteria for measurement of ultrashort PFAS from an EPA 1633 extract indicating suitability of the method for the measurement of these PFAS as an extension of a typical target PFAS method measuring from chain length C4 onwards. Results from the wastewater pilot are ongoing and will be presented.
MON-PM1-A4  Sorption mechanisms and efficiency for a series of PFAS and PFAS precursors by different activated carbon sorbents

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Introduction: Drinking water is considered one of the main sources of human exposure to PFAS. Authorities are introducing stricter guidelines and recommendations regarding the safe level of PFAS in drinking water and other exposure media, as knowledge of their toxicity and exposure considerations evolves. However, conventional water treatment processes are ineffective in removing PFAS, necessitating advanced treatments such as nanofiltration, reverse osmosis, and activated carbon sorption (Banks et al., 2020). Granular activated carbon (GAC) is widely used due to its cost-effectiveness and high contaminant removal efficiency (Militao et al., 2021). Given the stricter advised safety levels, improved environmental monitoring and water treatment for PFAS are essential. Available studies mostly focus on the removal of the well-known and now banned PFOA and PFOS by GAC, and in addition often use high concentrations that are not environmentally relevant. This work aims therefore to study sorption of a wide mixture of 31 PFAS in a batch set-up to a series of three GAC differing in porosity and micro- and mesopore volumes and including thermally reactivated GAC. We used environmentally relevant concentrations in a real drinking water matrix. We aimed to highlight the differences in sorption mechanism for different PFAS classes, providing insight into the role of the chain length, functional groups and branching of the PFAS on the sorption.

Method and material: Three bituminous coal-based GAC F400, reactivated R-F400, and carbon sorbent SRD (with higher percentage of mesopores as compared with F400) were investigated in a batch experiment of 500 mL tap water spiked with a mixture of 31 PFAS (12.5 µl; 0.2 ng/µl), with 5 ± 0.1 mg sorbent. Samples were put in the shaker for 1h, 5h, 24h, 96h, 10 days, all in triplicate. After the allotted time, the sorbent was removed, and solid phase extraction (SPE) was performed on the aqueous samples.

Results and Discussion: The removal of PFCA (C4-C12) and PFSA (C5-C10) increase significantly with chain length, indicating that hydrophobic interactions play a dominant role in their sorption. SRD initially showed lower removal compared to F400 and R-F400, but higher removal after longer exposure. No clear relationship between removal rates and chain length was observed for PFAS precursors PFEA (C4-C10), sulfonamides (C4-C8), and sulfonamide acetic acids (C8). Short chain PFAS precursors generally exhibited higher removal than short chain PFCA and PFSA. The sorption of PFAS precursor classes to GAC appears to be driven by electrostatic interactions rather than hydrophobic interactions related to chain length. There was no significant difference in removal between linear and branched isomers of PFHxS on all tested GAC. However, both sulfonamide acetic acids showed a significant difference in removal, with higher removal observed for the branched counterparts. Transforming micropores to mesopores through GAC reactivation did not result in significant differences in removal, although some variations were observed in the removal efficiencies over time. Based on the low removal of PFAS from environmentally relevant concentrations of drinking water using GAC, it is clear that urgent attention is needed to improve carbon sorbents for effective elimination of the PFAS and ensure that PFAS concentrations remain below safe levels.

Acknowledgements: Funding received from the European Union’s Horizon 2020 research and innovation programme under the Marie-Skłodowska-Curie grant agreement No 860665 (PERFORCE3 ITN project).

Reference:
**MON-PM1-A5** Decontamination of PFAS contaminated fire suppression system pipes – treatment verification with time-of-flight elastic recoil detection (ToF-ERD)

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**Introduction:** Per- and polyfluoroalkyl substances (PFAS) are a group of manmade chemicals which are known for their persistency, toxicity, bioaccumulative potential as well as ubiquitous low level distribution in environmental matrices, wildlife, and humans. Recently, the largest ever substance ban has been proposed by 5 EU member states aiming to restrict PFAS as an entire group of chemicals under REACH. Major sources of PFAS entering the environment are usage of PFAS-containing foam including aqueous-film-forming foam (AFFF). Fluorinated firefighting foams are used at fire-fighting training facilities, airports and in fire suppression sprinkler systems in industrial facilities. Targeted PFAS analysis does not account for the vast majority of organic fluorine present in moderns AFFFs. Numerous studies identified a wide range of PFAS including legacy perfluorocarboxylic and perfluorosulfonic acids are components of foams manufactured using electrochemical fluorination. Modern foams manufactured by fluorotelomerisation are dominated by precursor-PFAS which may be zwitterionic, cationic and anionic. The precise composition of PFAS in many fluorinated foams remain proprietary, but several recent publications have elucidated the structure of the PFAS they contain. Since new PFAS guidelines and restrictions are implemented, industries are being pushed to fulfill stricter regulatory limits. However, simply changing to Fluorine Free Firefighting (F3) Foams will likely be insufficient since PFAS adsorb to inner walls of sprinkler system pipes and potentially leach out into PFAS-free foam alternatives also known as rebound effect, because PFAS self-assemble to form supramolecular structures on solid surfaces. Therefore, it will be essential to perform cleaning procedures of fire suppression systems before changing to F3 foams. More importantly, for confirmation of successful decontamination, comprehensive measurement of PFAS on the inner surfaces of sprinkler systems is required. Analytical methods which comprehensively detect PFAS on surfaces can be applied during decontamination to avoid PFAS rebounding into F3 foams.

In this study, we aim to investigate removal efficiencies of several treatment solutions by soaking decommissioned pipe sections of fire-fighting suppression systems contaminated with PFAS by assessment of total fluorine mass present in the cleaning solution. Besides performing the actual purification, this study furthermore aims to visualize and compare fluorine mass on the surfaces before and after treatment using time-of-flight elastic recoil detection (ToF-ERD).

**Materials and Methods:** 10 cm long sections of decommissioned stainless steel sprinkler system pipes will be filled with tap water and aqueous solution with 10% and 20% butyl carbitol (BC). Each solution will be tested in triplicates at room temperature, 40 °C and 70 °C. Rinse solution will be changed after 12, 24, 72 and 168 h. Rinse solutions will be analyzed for PFAS concentration via LC-MS/MS by a targeted analysis and total oxidizable precursor assay (TOP-Assay). After 168 h, pipes will be left to rest empty for 4 weeks and soaked in tap water and PFAS free foam for one week thereafter rebound effects will be assessed. Surfaces of pipes will be analyzed with ToF-ERD before, during and after treatment to determine treatment efficiency and account for PFAS mass left on surfaces after treatment to verify treatment efficiency.

**Results:** To date, preliminary results regarding the PFAS contamination on inner surfaces on sprinkler system pipes, via aggressive PFAS extraction by sonication in methanol and qualitative measurement of PFAS assemblies on surfaces via scanning electron microscopy (SEM) exist. Aggressive methanol extraction revealed ΣPFAS concentration starting at 5.5-7 µg/cm² after targeted analysis, SEM analysis indicated clear differences in surface structure between pristine stainless steel and inner surfaces of sprinkler system pipes.

**Discussion and Conclusion:** The results of this work will contribute to finding effective solutions for purification of PFAS-contaminated fire suppression systems and contaminated metal surfaces. The experimental setup will allow to verify under which conditions PFAS assemblies will dissolve most efficiently and guide future application scenarios. Furthermore, surface analysis after treatment will rule out inefficient treatments.

**Acknowledgments:** This project has received funding from the European Union’s Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 860665.
MON-PM1-B1 Semi-quantitative identification of known and novel contaminants in indoor dust by ion-mobility high-resolution mass spectrometry and estimation of risks for human exposure.

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Introduction: The indoor environment has a major contribution to human exposure to various environmental contaminants. Thereby, the ingestion and inhalation of or the dermal contact with indoor dust represent main exposure routes which are especially relevant for toddlers due to crawling behavior and frequent hand-to-mouth contact. Several studies have identified various contaminant classes in dust by employing both quantitative targeted and suspect screening approaches [1,2]. While targeted studies provide unequivocal identification and quantitative data for known contaminants, suspect screening studies allow the simultaneous identification and detection of compounds which would be overlooked within targeted approaches. However, results obtained by suspect screening often lack quantitative information hampering the interpretation of results and the estimation of potential human exposure.

The present study describes a suspect screening analysis by liquid chromatography-ion-mobility high resolution mass spectrometry (LC-IM-HRMS) of indoor dust samples collected in Belgium. The development of a semi-quantification approach was used to estimate potential human exposure to the identified contaminants.

Materials and Methods: Indoor dust samples (n=46) were collected at 40 different locations in Flanders, Belgium. Dust samples were extracted with n-Hex:Acetone (1:1; v/v), fractionated by Florisil and analyzed by LC-IM-HRMS. The obtained data were matched with a suspect list containing > 4000 compounds covering various contaminant classes. Obtained IM derived collision cross section (CCS) values for the identified suspects were matched against previously established reference values and m/z-CCS trendlines [3] to increase identification confidence. Additionally, identified suspects were semi-quantified using calibration curves of several contaminants for which reference standards were available. For each suspect compound, a calibrant showing the highest similarity in structure and retention time was selected. From the (semi-)quantified concentrations, estimated daily intakes (EDI) and hazard quotients (HQ) based on different exposure models were calculated.

Results: More than 60 contaminants were identified with confidence levels (CLs) ranging between 1 and 3 according to the system of Schymanski et al. [4] whereby experimental CCS values added identification confidence, especially for compounds assigned with CL3 [5]. (Alternative) plasticizers and organophosphate esters (OPs) were the two classes with the most identified compounds. Results included several known contaminants, such as diisononyl phthalate (DINP) or tributoxyethyl phosphate (TBOEP), confirming their ubiquitous occurrence in the indoor environment. Additionally, numerous novel contaminants were identified, such as decyl nonyl phthalate and decyl undecyl phthalate, whose partially uneven numbered and different substituents were confirmed by characteristic fragments. These novel phthalates showed high detection frequencies (> 80%) and high median and maximum concentrations (e.g., 2 and 600 µg/g for decyl nonyl phthalate, respectively) comparable to the values observed for known phthalates. While the calculated HQs did not indicate a potential exposure risk for any of the phthalates, the HQs obtained for novel phthalates were in the same order of magnitude as for the most abundant known plasticizers such as di(2-ethylhexyl) phthalate.

The presence of one emerging OP, (bis(2,4-di-tert-butylphenyl) pentaerythritol diphosphate (BDTPDP)), recently described for the first time by Wang et al. [6], was confirmed in the Flemish dust. Additionally, two not previously described novel OPs (bis(2-butoxyethyl) butyl and didecyl butoxyethoxyethyl phosphate) were identified based on characteristic fragmentation spectra, showing DFs of 45.7 and 4.3%, respectively.

Discussion and Conclusion: The described workflow allowed the identification of novel phthalates and OPs whose high semi-quantified concentrations indicate that current targeted methods focusing only on known contaminants might underestimate human exposure. The combination of targeted and suspect screening approaches implementing IM derived CCS values as an additional identification parameter provides a comprehensive analytical platform for future qualitative and quantitative studies of dust samples. Further studies on relevant human exposure biomarkers including these novel compounds are needed for a more comprehensive assessment of exposure to these compound classes.

MON-PM1-B2 Application of Perfluorocarboxylic Acids Detected Environmentally at High Frequency as Retention Indices of Contaminants of Emerging Concern in Simultaneous Screening Analysis Using LC-QTOF/MS

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Introduction:
Chromatographic retention time (RT) is important information for improving identification level in screening analysis using a high-resolution mass spectrometry (HRMS). However, especially in liquid chromatography (LC), RT is highly dependent on the type of instrument and on the application environment by each research institution. Moreover, there are day-to-day fluctuations even in the same instrument. Therefore, it has been common for each research institution to add stable isotope-labeled standards as retention time indices (RIs) and to predict the RT of the substance to be measured based on the RT of the RIs to improve those identification levels. On the other hand, it is not always possible to add same RIs in all the research institutions while the analytical conditions of HRMS are desired to be uniformed for its data application to screening analysis with shared databases. In this study, we aimed to improve the quality of screening analysis using LC-QTOF/MS, which is high-resolution and is applicable for comprehensive analysis of environmental water samples. We focused on perfluorocarboxylic acids (PFCAs) with different carbon chain lengths as they are detected with high sensitivity by LC/MS and are reported to be detected at high frequency in the water environment. We investigated the applicability of environmentally detected PFCAs as RIs so that there is no requirement for the addition of standard substances.

Materials and Methods:
Measurements were conducted in MS² mode on a LC-QTOF/MS (Xevo G2-XS QToF, Waters). ACQUITY UPLC HSS T3 (1.8 µm, 2.1 × 150 mm, Waters) was used as the separation column of the LC and the flow rate was set at 0.4 mL/min when measuring environmental samples. The 8 substances selected as candidates of RIs were perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluorohexanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), and perfluorododecanoic acid (PFDoDA). In this study, 5 examinations were conducted. Firstly, environmental water samples were analyzed to understand the detection frequencies of PFCAs in the environment. During the winter of 2021, total of 25 river samples were collected from 11 prefectures in Japan (Gifu Pref. (n=15), Fukushima Pref., Gunma Pref., Saitama Pref., Mie Pref., Shiga Pref., Osaka Pref., Nara Pref., Kagawa Pref., Kumamoto Pref., Kagoshima Pref. (n=1, respectively)). Secondly, standard substances of the 8 PFCAs were analyzed to investigate the analytical behavior of PFCAs under different flow rates. In this examination, ACQUITY UPLC HSS T3 was used as the separation column of the LC, and the flow rate was changed with 4 different conditions (0.1, 0.2, 0.3 and 0.4 mL/min).

In this equation $RT_{Target-RI}$ represents predicted RT of a target substance using RIs, $RT_{Target-DB}$ represents predicted RT of a target substance registered in the database, $RT_{Before-sample}$ represents RT of a RI eluted just before a target substance detected in environmental samples, $RT_{Before-DB}$ represents RT of a RI eluted just before a target substance registered in the database, $RT_{After-sample}$ represents RT of a RI eluted just after a target substance detected in environmental samples, and $RT_{After-DB}$ represents RT of a RI eluted just after a target substance registered in the database. The absolute difference between measured RT and predicted RT ($\Delta RT$) was calculated both in the case with $RT_{Target-RI}$ and that with $RT_{Target-DB}$ and the results were compared.

$RT_{Target-RI} = RT_{Target-DB} \left\{ \frac{RT_{Before-sample}}{RT_{Before-DB}} + \frac{RT_{After-sample}}{RT_{After-DB}} \right\}/2$
Finally, we applied the calculation of $RT_{\text{Target-RI}}$ to a group of target substances detected in environmental samples applying PFCAs as RIs, which were detected in the corresponding samples. In September and November 2022, total of 48 environmental water samples were collected from rivers and outlets of wastewater treatment plants (WWTPs) in 5 prefectures in Japan (Gifu Pref. ($n=24$), Tokyo Pref. ($n=6$), Saitama Pref. ($n=4$), Chiba Pref. ($n=2$) and Kanagawa Pref. ($n=12$)). The substances measured were 102 contaminants of emerging concern (CECs) included in the previously reported database. The detection of them in each sample was judged based on the mass error tolerance of 20 ppm for the precursor ion and the mass error tolerance of 50 ppm for the product ions. Detection of a substance was determined when spectrum of the registered precursor ion in the database was found and when spectrum (or spectra) of more than one of the registered product ions were found. The detection was confirmed only when $|\Delta RT|$ by $RT_{\text{Target-RI}}$ was within 1.0 min. As the method of sample pre-treatment, collected samples (500 mL) were filtered by glass fiber filter papers (GF/B, 47 mm diameter, Whatman) to separate the dissolved-phase and particulate-phase samples. Dissolved-phase samples were pre-treated by solid-phase extraction using Oasis HLB Plus and Sep-Pak AC2 Plus (Both Waters). Particulate phase samples were freeze-dried and pre-treated by sonication with methanol and acetone. After concentrating with nitrogen purging, treated samples were reconstituted in 1 mL or 0.5 mL of methanol and ultrapure water (1:1, v/v) and filtered by vial with filtration function (Mini-UniPrepTM G2, 0.2 µm, PP, Whatman) before instrumental analysis.

Results and Discussion:
Detection frequencies of 8 PFCAs from 25 river samples are shown in Fig.1. The detection frequencies of PFHxA, PFHpA, PFOA, and PFNA were 100%. These were mainly detected in dissolved phase samples. The detection frequencies of PFPeA, PFDA, PFUnDA, and PFDoDA were 44% (11 out of 25 samples), 52% (13 out of 25 samples), 48% (12 out of 25 samples), and 20% (5 out of 25 samples), respectively. PFDA, PFUnDA, and PFDoDA were detected more frequently in particulate phase samples, and PFDoDA were only detected in particulate phase samples. These can be utilized as RIs when detected in at least 1 sample while more PFAAs are also recommended to be used to secure the number of available RIs. Comparison of RTs of 8 PFCAs at different flow rates is shown in Fig.2. At all flow rates, the substances with shorter carbon chain lengths eluted earlier, and the RTs shortened almost inversely related with increasing the flow rates. Even at the highest flow rate of 0.4 mL/min, each analyte was detected at approximately every 1.0 min, and their order of elution was not changed under different flow rates. Comparison of RTs of 8 PFCAs in different separation columns is shown in Fig.3. Inertsil ODS-4 HP showed almost the same RT with ACQUITY UPLC HSS T3. ZORBAX RRHD SB-C18 eluted all substances approximately 1.5 min faster than ACQUITY UPLC HSS T3. AcclaimTM RSLC 120 C18 showed a similar trend, eluting all substances approximately 1.0 min faster than ACQUITY UPLC HSS T3. The elution characteristic of each column was not remarkably different, while the order of RT for each substance was not changed. It was possibly due to the similar structure of PFCAs with just different carbon chain lengths. In consideration with the high detection frequency from environmental samples and the relatively stable analytical characteristics, PFCAs were considered one of the suitable substance groups to be used as RIs. Fig.4 shows the comparison of RT prediction of environmental samples between the results with and without using PFCAs as RIs. For substances detected at ESI(+), 2 out of 15 substances (erithromycin and clarithromycin) indicated no decrease in $|\Delta RT|$, while the other 13 substances indicated decrease in $|\Delta RT|$, ranging from 0.24 to 0.73 min. For substances detected at ESI(-), all 7 substances indicated decrease in $|\Delta RT|$, ranging from 0.34 to 0.77 min. The $|\Delta RT|$ was also decreased for substances eluted at outside the range of the RTs of 8 PFCAs.
This improvement of RT prediction reduced the false negative detection due to RT inconsistency. Under the detection criteria in this study, false negative detection was occurred at 27.3% of the target substances (6 out of 22 substances) when $RT_{Target-DB}$ was applied. On the other hand, the numbers of false negative detection were reduced to 0 when $RT_{Target-RI}$ with environmentally detected PFCAs was applied. This shows the effectiveness of RT prediction by PFCAs detected in environmental samples. The result of screening analysis of 102 substances and those detection frequency in environmental water samples ($n=48$) are shown in Fig.5. Only the analytical data of dissolved phase samples was demonstrated in this abstract. As a result of the screening analysis, a total of 43 substances was detected in at least one sample. The other 59 substances were not detected in any samples. 15 substances such as PFOS and crotamiton were detected at over 80% detection frequency, and these substances were considered to be high monitoring priority. In particular, telmisartan, caffeine, and bromacil were detected at 100% detection frequency. PFOS was detected at a high frequency, in spite of PFOS is regulated by the Stockholm Convention on Persistent Organic Pollutants[3], and is also regulated in Japan. The reasons were considered to be the persistence of PFOS[3] as well as its unintentional formation from precursors[3]. Crotamiton and telmisartan are said to be difficult to be removed in WWTPs[4,5], therefore these were detected at many sites in this study. On the other hand, the removal efficiency of caffeine by wastewater treatment processes is reported to be high[6], but it was detected at all sites including treated wastewater in this study. It was also detected at several sites in Japan at previous study[7].
It was suggested that the consumption and the loading of caffeine to WWTPs are tended to be high so that it remains in treated wastewater and in the water environment. Although screening analysis does not provide sufficient quantitative evaluation of individual target substances, it provides the overview of occurrences of various substances in the environment more quickly and broadly than target analysis as shown above. This can evaluate the monitoring priority and the needs of further investigation including quantification by target analysis and evaluation of toxicity, without inputting information before instrumental analysis.

Conclusions:
We investigated the applicability of PFCAs as RIs in order to improve the quality of screening analysis in this study. In consideration with the high detection frequency from environmental samples and the relatively stable analytical characteristics, PFCAs were considered one of the suitable substance groups to be used as RIs. It was also confirmed that RT prediction using PFCAs as RIs can reduce false negative detections in the screening analysis. Therefore, PFCAs detected in environmental samples were considered to be applicable as RIs without addition of internal standards or surrogate substances, making the screening database more versatile. PFCAs as the RIs were applied to the screening analysis of 102 CECs in 48 environmental water samples, resulted in the detection of 43 substances. The screening analysis identified 15 substances of high monitoring priority, such as caffeine, telmisartan, bromacil, clarithromycin, and PFOS.
Screening and Identification of Novel Contaminants

E. Schymanski & B. Le Bizec

MON-PM1-B2  Application of Perfluorocarboxylic Acids Detected Environmentally at High Frequency as Retention Indices of Contaminants of Emerging Concern in Simultaneous Screening Analysis Using LC-QTOF/MS

Acknowledgments:
This study was supported by the Environment Research and Technology Development Fund (JPMEERF20215G01) of the Environmental Restoration and Conservation Agency provided by Ministry of the Environment of Japan, and MEXT/JSPS KAKENHI Grant Number JP 22KK0058. We also appreciate Mr. Tatsunori Kimura of Tohoku Ryokka Kankyo Hozen Co., Ltd. for his support in instrumental analysis.

References:
MON-PM1-B3 Analytical strategies to confirm the presence of emerging PFASs in blood samples without analytical standards

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Introduction:
Since the 1950s, per- and polyfluorooalkyl substances (PFASs) have been extensively used in industrial and commercial applications due to their attractive properties, such as their thermal and chemical stabilities and their amphiphilic nature1. Due to their inherent stability and widespread use, these compounds are found and prevail in all environmental matrices. However, the presence of these compounds in the environment, water, and food is of concern as toxicological studies have demonstrated that PFASs may be related to several health issues such as thyroid disorders or cancers2. Nevertheless, only a few of these substances are regulated, such as perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA), which have been included in the annexes of the Stockholm Convention. In response to growing concerns about PFOS and PFOA, major manufacturers voluntarily discontinued production of these and related substances, in the early 2000s3. As a result, alternative PFASs have emerged and have been introduced to the market. Chloro-perfluoropolyether carboxylates (Cl-PFPECA) are one of the classes of emerging compounds3,4. They have been detected in New-Jersey soil samples downwind of two PFAS manufacturing facilities (Solvay and DuPont/Chemours)5, and in surface water and groundwater surrounding them6. Both facilities have been participating in the PFOA Stewardship program and a mixture of Cl-PFPECA oligomers, also called congeners in the related literature, was implemented as processing aid by Solvay7 and reported in a product evaluation by the European Food Safety Authority (EFSA), at their previous conditioning with 2 ml of methanol and 2 mL of water. The supernatant was loaded onto an Oasis WAX SPE cartridge (3 cc, 60 mg, 30 µm), previously conditioned with 2 mL of methanol and 2 mL of water. Two milliliters of 5% formic acid in water were added to 1 mL of serum. The acidified sera were sonicated for 15 minutes in 10mL clot activator tubes (BD Vacutainer MD) in Italy. Four water samples were prepared: the discharge water sample, a blank and groundwater surrounding them4. Both facilities have been participating in the PFOA Stewardship program3 and a mixture of Cl-PFPECA congeners in the discharge water of the Solvay facility located in Spinetta Marengo, Italy, and in the blood of some inhabitants of this region. Five congeners have already been identified in water sample from the Bormida River, downstream of this Solvay facility7. At the time we performed this study during summer 2022, as in the articles mentioned3,6, no ClPFPECA analytical standard was available. The major challenge of this work was therefore to conclusively identify the congeners with sufficient confidence. The target confidence level was at least 3 (i.e., tentative candidate structure or class of structures), as defined by conventional HRMS identification confidence context8,9. To this end, liquid chromatography coupled with high-resolution mass spectrometry was used for the analyses of water and blood samples and the results were supported by comparison with the literature data. This work is one of the first report published to assess the presence of Cl-PFPECA in human blood of non-occupational worker from the plant, except for a few retired former workers10.

Materials and Methods:
As a reference, a mixture of native standards (PFAC-MXC) was purchased from Wellington Laboratories, Inc (Ontario, Canada), and consisted of C4-C14, C16, and C18 perfluoroalkyl carboxylic acids (PFCAs), and C4-C10 and C12 perfluoroalkane sulfonic acids (PFSAs). The water sample analyzed in this study was collected directly from the discharge channel of the Solvay facility at Spinetta Marengo, Alessandria, Italy in March 2022, and diluted with groundwater. The study was performed on a cohort of 30 volunteers, living in Spinetta Marengo, close to the Solvay plant, some of them being former employees of this factory. Blood samples were collected in 10mL clot activator tubes (BD Vacutainer MD) in Italy. Four water samples were prepared: the discharge water sample, a blank of unspiked Milli-Q water, another blank of congener mixture, and a reference sample with spiked tap water. The filtered water was loaded onto an Oasis WAX SPE cartridge (6 cc, 150 mg, 30 µm), previously conditioned with successively 4 ml of 0.2% ammonium hydroxide in methanol, 4 mL of methanol and 4 mL of water. The column was then washed with 4 mL of 20 mM ammonium acetate, followed by 4 mL of methanol. The compounds were eluted with 4mL of 0.2% ammonium hydroxide in methanol. The eluate was filtered with a 0.2 µm nylon syringe filter (Fischer Scientific, Hampton, NY, USA) and evaporated to dryness at 30 °C under gentle flow of nitrogen. The residue was finally reconstituted in 250 µL of a 95/5 (v/v) methanol/water mixture and transferred to an injection vial. For the blood samples, in addition to the 30 blood samples from Italy, unspiked and spiked control sera were prepared and analyzed. The protocol used for the preparation of blood samples was based on a paper by the toxicology laboratory of the University of Liege12, with some adaptations. First, sera were obtained after centrifugation of coagulated blood samples. Two milliliters of 5% formic acid in water were added to 1 mL of serum. The acidified sera were sonicated for 15 minutes and centrifuged at 5000 rpm for 10 minutes. The supernatant was loaded onto an Oasis WAX SPE cartridge (3 cc, 60 mg, 30 µm) previously conditioned with 2 mL of methanol and 2 mL of water.
The cartridge was then washed with 1 mL of 2% formic acid in water, followed by 1.5 mL of methanol. Analytes were eluted with 2 × 2 mL of ammonium hydroxide 2% in methanol. The same steps as for the water samples were applied to the eluate except that it was reconstituted in 80 µL of a 95/5 (v/v) methanol/water mixture. Unspiked and spiked control sera (8 µL of PFAC-MXC in 1 mL) were also analyzed. No internal standard was added to the samples, the main objective being to confirm or deny the presence of CI-PFPECA congeners. Chromatographic separation was performed on an Acquity I-Class UPLC system (Waters, Milford, MA, USA) using a Acquity BEH C18 column heated to 45 °C (2.1 × 150 mm × 1.7 µm particles) (Waters, Milford, MA, USA). Chromatographic separation was conducted on an injected volume of 5 µL for the water samples, and 10 µL for the blood samples. The flow rate was 0.2 mL/min with a binary mobile phase gradient of solvent A (water + 0.1% formic acid) and solvent B (acetonitrile). The gradient was adapted from the literature13 and started at 20% B and increased linearly from 20% to 40% (0-0.5 min); remained constant for 1 min (0.5-1.5 min); increased linearly from 40% to 100% for 10 min (1.5-11.5 min); remained constant at 100% until 19.5 min; decreased from 100% to 20% (19.5-20 min) and was kept constant at 20% during 9 min to recondition the column for the next analysis. After each sample injection, 1 µL of a blank mixture of water/MeOH (95/5 V.Vv) was injected to prevent carryover. The LC was coupled to a Q-Exact Orbitrap high-resolution mass analyzer (Thermo Fisher Scientific, Waltham, M, USA) equipped with an electrospray source operating in the negative mode. The MS/MS analyses were performed using the data-dependent acquisition mode and scan settings were based on the literature4. For data processing, Xcalibur (Thermo Fisher Scientific, Waltham, M, USA) and Skyline14 software were used. Data were analyzed manually, and structures were tentatively identified based on MS/MS data and comparison with literature data3,4,16.

3. Results:
Some homologs of the legacy PFCAs (C4-C11) and PFSAs (C4-C6, C8) were identified in the discharge water at the same retention times as in the tap water spiked with the PFAC-MXC mixture containing these analytes. For each PFCA homolog, [M-H]- and [M-H-COO]- ions were co-eluting and the mass accuracy on both ions was less than 3 ppm. In addition, MS/MS spectra obtained from PFCA [M-H]- and [MHCF2CO2]- ions displayed peaks compatible with [CF3[CF2]x]- ions within a mass accuracy range of 5 ppm. Five CI-PFPECA congeners (Figure 1) were tentatively identified in the discharge water sample, whereas they were not detected in the tap water and milli-Q water blanks.

The identified congeners corresponded to: e,p = 1,0; e,p = 0,1; e,p = 2,0; e,p = 1,1; and e,p = 0,2; and were eluted in the same retention time range as ClC4F9 and ClC6F13 PFCAs (POFA and PFNA). As for PFCAs, for each CI-PFPECA congener, another ion was found to co-elute with the [M-H] ion. This ion could correspond to the loss of one CF2CO2 unit from the [M-H] ion and was the predominantly observed ion. The mass accuracy on both [M-H] and [MHCF2CO2] ions was below 2 ppm for each congener. The natural abundance of chlorine was verified by evaluating the M+2/M isotopic ratio of the [M-H-CF2CO2]- ion (i.e., most intense ion) for each congener. For all five congeners, this ratio was compatible with the expected value of 32%.

Moreover, an ion with m/z equal to 200.9547, within a mass accuracy range of 5 ppm, was found at the same retention time as all five congeners (Figure 2). This mass-to-charge ratio is consistent with a compositional formula of [ClC3F6O]-, which could correspond to the [M-H-CF2CO2]- fragment of Cl-PFPECA e,p = 0,0. It is therefore coherent that this ion is a common ion between all congeners. Figure 2 also illustrates the chromatographic trace of the M+2 isotope due to the presence of the chlorine atom on this common ion.
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Finally, the MS/MS spectra of the \([M-H]\) and \([M-H-CF_2CO_2]\) ions of the \(e,p = 0,1\) and \(e,p = 0,2\) congeners were recorded in DDA mode, as well as the MS/MS spectrum of the \([M-H-CF_2CO_2]\) of the \(e,p = 1,1\) congener. The MS/MS spectra of the deprotonated \(e,p = 0,1\) and \(e,p = 0,2\) ions displayed one feature consistent with the loss of the \(CF_2CO_2\) unit and another with the common \([ClC_3F_6O]\) fragment within a mass accuracy range of 5 ppm. This common fragment was also detected in the MS/MS spectra of the \([M-H-CF_2CO_2]\) ions of these two congeners and of the \(e,p = 1,1\) congener. Finally, the MS/MS spectra of the two ions of congener \(e,p = 0,2\) displayed a peak coherent with the \([MHCF_2CO_2]\) ion that lost a fluoroether propyl unit \((CF_2CF(CF_3)O)\).

Legacy PFCAs \((C_4\text{ and }C_{7-10})\) and PFSAs \((C_{4-6}\text{ and }C_8)\) were detected in the analyzed blood samples, with a mass accuracy of 2 ppm on the deprotonated ions of PFCAs and PFSAs and on the \([M-H-COO]\) ions of PFCAs. MS/MS spectra of the most abundant homologs were recorded in DDA and compatible with the identified PFCAs and PFSAs. A slight retention shift was observed between the blood samples and the discharge water sample for these compounds. Therefore, based on the retention times observed for Cl-PFPECA congeners in the discharge water sample, their retention times in the blood sample analyses could be predicted. The coeluting \([M-H]\) and \([MHCF_2CO_2]\) ions of congener \(e,p = 0,1\) were detected in all 30 blood samples, within a mass accuracy range of 2 ppm. These ions were detected at the same retention time in all blood samples tested and their retention time were consistent with the ones observed in the discharge water sample. For the other four congeners identified in the discharge water, depending on their intensities, the \([M-H]\) ion could not be detected in the blood samples, but the more abundant \([M-H-CF_2CO_2]\) ion could be identified at retention time consistent with the discharge water sample. Furthermore, the expected 32% value of the \(^{37}Cl\) and \(^{35}Cl\) isotope ratio of this ion was observed for these four congeners in the samples with the highest Cl-PFPECA signal intensities. In addition, MS/MS spectra were acquired for all five congeners in these blood samples and the peaks identified were consistent with the fragments of these compounds in the 5-ppm mass accuracy range. With respect to these considerations, the \(e,p = 0,1\) congener was identified in all the 30 blood samples, while \(e,p = 0,2; e,p = 1,0; e,p = 1,1\) and \(e,p = 2,0\) congeners were identified in 20, 6, 27 and 27 samples, respectively. No Cl-PFPECA congeners were identified in the control serum samples.

Discussion:
The presence of the legacy PFOA and PFNA in the discharge water sample was used as a reference for the retention times. Indeed, the relative retention times of Cl-PFPECA congeners to PFOA and PFNA were available in the literature. Using a mobile phase gradient that went linearly from 20/80 ACN/H_2O with 0.1% formic acid to 90/10 ACN/H_2O with 0.1% formic acid, the observed elution order was as follows: CI 1,0 < PFOA < CI 0,1 < PFNA < CI 2,0 < CI 1,1 < CI 0,2. This elution order is the same as the one observed in Figure 1, increasing the confidence in the identification of these congeners in the discharge water sample. Furthermore, the five congeners identified in the discharge water are the same as those identified in the Bormida River, downstream of the Solvay plant of Spinetta Marengo, where the analyzed discharge water was collected. Furthermore, the relative intensities of the five congeners are similar in the discharge water sample and the river water sample. Therefore, even though no analytical standard were available for the CI-PFPECA congeners, consistency with literature data increases the confidence level in their identification, in addition to verification of the isotopic pattern due to the chlorine atom and identification of several distinctive fragments in the MS spectra (in-source fragmentation) and DDA MS/MS spectra. In the literature, the \([M-H-CF_2CO_2]\) has also been reported as the major observed ion in the MS spectra of each congener. In conclusion, confidence level 3 (i.e. tentative structure in the identification of the 5 congeners of Cl-PFPECA in the discharge water sample is reached, and the decisional tree used to achieve this confidence level is represented in Figure 3. However, the exact structure of the congeners cannot be determined as some Cl-PFPECA elute as split peaks, which may reflect the presence of structural isomers. For instance, the chlorine atom could be on the ultimate or penultimate carbon atom, isomerization within the fluoroether propyl unit(s), and group regioisomerism.
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between the ethyl and propyl groups could be likely\(^3\). For the blood samples, confidence level 3 is also reached for the identification of the same five congeners as in the discharge water sample. Indeed, although the deprotonated ions could not be identified for all five congeners in each sample, the detection of the [M-H-CF\(_2\)CO\(_2\)]\(^-\) ions at consistent retention times between the blood and the water samples was sufficient for identification. The latter was confirmed by verifying the isotopic pattern due to the chlorine atom on the [MH\(_2\)CF\(_2\)CO\(_2\)]\(^-\) ions. Moreover, the same distinctive ions as in the discharge water sample could be identified in the MS (probably in-source fragmentation) and DDA MS/MS spectra of the most contaminated samples.

Figure 3: Decisional tree summarizing the selection and verification criteria that were used to assess the presence of the Cl-PFPECA congeners in the discharge water sample.

Conclusions:
The focus of this study was to identify emerging Cl-PFPECA congeners in a sample of wastewater from the Solvay plant in Spinetta Marengo, Italy, and in blood samples from residents of the area near this facility. Despite the unavailability of their analytical standards, five Cl-PFPECA congeners could be identified within the third confidence level as described in the conventional HRMS identification confidence context. This could be achieved by performing UPLC-HRMS/MS analyses in data-dependent acquisition mode. Congener identification was supported by the detection of distinctive ions in the MS and MS/MS spectra and by the observation of the isotopic pattern due to the presence of a chlorine atom. The identification was also supported by comparison with data available in the literature (i.e., elution order relative to legacy PFCAs) of the identification of Cl-PFPECA in soil and water samples. Nevertheless, the exact structure of the congener could not be accurately determined, and the split chromatographic peaks could indicate the presence of several isomers. Coupling an ion mobility spectrometry method with LC-MS could increase the separation power and assist in the separation of potential isomers of Cl-PFPECA congeners. In addition, degradation products (e.g., hydrohalogenated H-PFPECA\(^{1,16}\)) of identified Cl-PFPECA congeners could be sought in the blood samples to monitor their degradation pathways.

Acknowledgments:
The Q-Exactive mass spectrometer was funded by ERDF and the Walloon Region. We would like to acknowledge the 30 volunteers who participated to the blood sampling for the study.

References:
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6. EFSA Panel on food contact materials, enzymes, flavourings and processing aids (CEF), 2010, Scientific Opinion on the safety evaluation of the substance perfluoro acetic acid, α-substituted with the copolymer of perfluoro-1,2-propylene glycol and perfluoro-1,1-ethylene glycol, terminated with chlorohexafluoropropyleoxy groups, CAS No. 329238-24-6 for use in food contact materials., EFSA J., 8(2),1519


MON-PM1-B4 PFAS analysis in the blink of an eye using DART-MS

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VITO, Boeretang 200, 2400, Mol, Belgium

Introduction: The increasing number of studies concerning PFAS show that PFAS have become a widespread environmental problem showing up in a variety of matrices. Governmental and public interest in the PFAS issue is also increasing leading to a growing number of samples and sample types that are subjected to analysis. The search for faster, cheaper, and more comprehensive methods is bigger than ever.

We have implemented Direct Analysis in Real Time Mass Spectrometry (DART-MS) to be able to handle high amounts of samples requiring no sample prep and deliver both targeted and untargeted results. Rapid data collection in combination with customized databases and expert judgement are now a powerful new tool for PFAS analysis.

Materials and Methods: DART-MS is an ambient ionization technique that allows direct mass spectrometry analysis of solid and liquid materials without preliminary sample prep or chromatography. Additionally, since the DART-MS is coupled to a high-resolution Mass Spectrometer both targeted and untargeted analysis can be performed. First reference standards of PFAS compounds were analyzed to study their behavior in the DART ionization source and to build an in-house database. As a proof-of-concept we have studied different types of materials, such as (ground)water, seafoam, soil, dust and different PFAS classes.

Results: The analysis of a wide variety of samples show that DART-MS is able to detect a significant number of PFAS, ranging from ultra-short chain PFAS like TFA, up to perfluorinated acids with masses above 1000 Da as intact molecules. Other compounds like DiPAP for example are unstable at the high DART-MS source temperatures and degrade before analysis. Therefor only fragments of these compounds could be measured which in the database can be attributed as fragments, potentially indicating the presence of the parent molecule.

The analysis of different environmental materials shows that PFAS can be detected in all of these sample types, showing the versatility of the technique. Moreover, using the high-resolution data, DART-MS has allowed detection of unknown PFAS, which later could be verified using LC-HRMS.

Discussion and Conclusion: We show that DART-MS can be used for fast screening of a high variety of solid and liquid samples both in a targeted and untargeted way. This allows fast selection of interesting samples for large screening campaigns, indication of highly contaminated samples allowing adjusted sample prep and detection of unknown PFAS.

References: Direct analysis in real time-a critical review on DART-MS, Gross, JH Analytical and bioanalytical chemistry 406 (1), pp.63-80
MON-PM1-B5  Could Sentinel Animal Species Be Used to Early Identify Halogenated Chemicals of the Human Food Exposome?

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Introduction: Diet is a significant source of exposure to well-known persistent organic pollutants (POPs) for humans, particularly food from animal origin (FFAO). The polyhalogenation, a common characteristic of POPs, confers them persistency and hydrophobicity. Furthermore, because of the increasing regulatory pressure against POPs[1], alternative substances have emerged for similar industrial applications. Some of them may be released into the environment and may enter the human food chain. Describing precisely the human food chemical exposome for supporting risk assessment requires the identification of both known legacy contaminants and contaminants of emerging concern. However, the detection of emerging contaminants in foodstuffs or human tissues is limited by sensitivity and/or ethical issues. Resorting to sentinel animal species may allow these limitations to be lifted. Selecting species whose habitat and diet are similar to human appears relevant. This study aimed to confirm the use of sentinel animal species as an early warning to identify halogenated emerging contaminants.

Materials and Methods: Samples from animal species with an opportunistic diet and living in urban areas were collected in 2015, 2021 and 2022 in France. Gull eggs were sampled in colonies located in and close to urban areas along the French coasts of the Channel Sea, the Atlantic Ocean (Larus argentatus) and the Mediterranean Sea (Larus michahellis). Pigeon eggs (Columba livia) were sampled in Paris, Tours and Montpellier. Adipose tissues and livers were sampled from pigeons euthanized by the municipal department of Tours and from rats (Rattus norvegicus) collected after death by the municipal department of Montpellier. FFAOs samples were selected on the basis of the French food consumption habits and purchased in 2022 in Nantes. Samples were freeze-dried and microwave extracted. Lipid extracts (200 mg) were purified by gel permeation chromatography. Eluates were analysed using GC- and LC-HRMS (full scan mode). Data treatment was performed with HaloSeeker software on the most intense signals[2]. Halogenated signals revealed in sentinel samples were searched in FFAO samples using Skyline software.

Results: Several halogenated signals were detected in both sentinel samples and FFAOs. Legacy POPs such as PCBs, PBDEs and hexachlorobenzene (HCB) were detected and tentatively identified by GC-HRMS. TBBPA, dichlorophenol (DCP) and monobromophenol (MBP) isomers were detected and tentatively identified by LC-HRMS. For several contaminants, highest levels were detected in sentinel matrices (Table 1). For sentinels, all compounds were detected at higher levels in eggs, except DCP (livers).

Table 1: Highest levels normalized by external standard for tentatively identified compounds. In brackets, detection frequencies for FFAOs.

<table>
<thead>
<tr>
<th></th>
<th>Sentinels</th>
<th>FFAOs</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_{12}H_{3}Cl_{7} (PCB)</td>
<td>52</td>
<td>0.6 (51%)</td>
</tr>
<tr>
<td>C_{12}H_{4}Cl_{8} (PCB)</td>
<td>32</td>
<td>0.3 (33%)</td>
</tr>
<tr>
<td>C_{12}H_{4}OBr_{6} (PBDE)</td>
<td>0.3 (.0%)</td>
<td>ND (0%)</td>
</tr>
<tr>
<td>C_{6}Cl_{6} (HCB)</td>
<td>5.3</td>
<td>357 (86%)</td>
</tr>
<tr>
<td>C_{14}H_{12}OBr_{6} (TBBPA)</td>
<td>3.4</td>
<td>8.2 (94%)</td>
</tr>
<tr>
<td>C_{6}H_{4}OCl_{2} (DCP)</td>
<td>19</td>
<td>5.6 (63%)</td>
</tr>
<tr>
<td>C_{6}H_{4}OBr (MBP)</td>
<td>4.2</td>
<td>28 (88%)</td>
</tr>
</tbody>
</table>

Discussion and Conclusion: PCBs and PBDEs appeared more concentrated in the sentinel samples than in the FFAOs, indicating a higher bioaccumulation. However, they are among the well-known legacy contaminants that are regularly monitored in FFAOs using targeted methods, so that the early warning does not apply. HCB and TBBPA were found at higher concentrations in the seafood than in the sentinel samples, which minimises the relevance of the sentinel samples for these other legacy contaminant. MPB was found at higher concentrations in seafood products than in sentinel samples. It has been described as halogenated natural compound responsible for the flavour of seafood[3]. In contrast, DCP was found in the sentinel samples and may be a metabolite of the pesticide 2,4-dichlorophenoxyacetic acid[4]. Further data mining revealed other interesting compounds, for which the structural identification remains a challenge. Finally, sentinel specimen were collected to reveal the human dietary chemical exposome. The link to the human food chain remains to be characterised, e.g. by analysing gastric contents or by comparison with colonies in remote areas.

Acknowledgements: Financial support: “Région Pays de la Loire, France”. Expertise and sample collection: T. Boulinier (CNRS, Montpellier), P. Bustamante (CNRS, La Rochelle), J. Gasparini (Sorbonne University, Paris), P. Gourlay (Oniris, Nantes) and the municipal departments of Le Havre, Lorient, Le Croisic, Les Sables d’Olonne, Tours and Montpellier.

References:
MON-PM1-C1  Results of the UNEP worldwide monitoring of persistent organic pollutants in biota, sediment and food products

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3Laboratroy of Dioxins, IDAEA-CSIC, Barcelona, Spain

Introduction:
Between 2016 and 2019 a large United Nations Environment Program (UNEP)/Global Environmental Facility (GEF) monitoring project was carried out with the aim to contribute data to the global monitoring plan (GMP) of the Stockholm Convention. A second objective was to evaluate the capacity of the participating laboratories in analyzing persistent organic pollutants (POPs) in samples of their own interest. This project also included monitoring of POPs in air (de Boer et al., 2023) and human milk (Fiedler and Sadia, 2021), which are not reported here. The Stockholm Convention GMP focuses on a selected set of so-called ‘core’ matrices, air, human milk, water, and human serum. Countries have, however, regularly expressed their interest in analyzing other matrices, such as food items, which are of more national interest, or sediments. It was therefore decided to offer the option for countries to select their own matrices. The samples collected should, after homogenization, be split into two parts, one to be analyzed by the countries themselves, one being sent for analysis to a one of the reference laboratories in this project. The additional advantage of this set-up was a comparison between analytical data. The POP concentrations found would also be useful to compare with POP levels in air, to find possible correlations or discrepancies. Twenty-five countries from all continents were involved in this study, most of them situated between 36°N and 36°S, with the exception of Mongolia (ca. 45°N) and Chile (Patagonia) (ca. 45°S).

Materials and Methods:
Table 1 shows a list of participating countries, together with the sample type analysed. On average countries could send ten samples to the reference laboratories. In practice, strong deviations occurred, due to logistics, insufficient quality control during shipment, and other reasons. A standard operating protocol was prepared to assist the countries in sampling and sample handling. All countries were asked to at least send one fish sample, just to have one common matrix. In total 197 samples were received and analysed. In some cases, countries, such as Brazil, had sent biotic matrices, but the transport was blocked by customs from entering the European Union and the samples could not be delivered to the reference laboratories. In other cases (e.g., Ecuador), the samples were shipped too late, and they could not be analysed before the final project deadline. The report focuses on organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs) and polybrominated diphenylethers (PBDEs). Per- and polyfluorinated alkyl substances (PFAS), polychlorinated dibenzodioxins and dibenzofurans, and dioxin-like PCBs were also analysed in these samples but are not included here (Fiedler et al., 2022, 2023).

For the Asian, Pacific and African samples, the sediment, soil, fish, beef, maize and pulse samples were homogenized and extracted with accelerated solvent extraction (ASE) with hexane:acetone (3:1, v:v) as extraction solvent. While the egg samples were extracted using the non-chlorinated solvents cyclohexane and 2-propanol. An aliquot was taken of the egg extraction to determine the total lipid (gravimetric analysis). The clean-up for the PBDEs and toxaphene was carried out over silica columns impregnated with sulphuric acid to remove residual matrix such as humic acids and lipids. In sediment/soil samples for PBDE analysis, removal of sulphur was performed by gel permeation chromatography (GPC), followed by a fractionation with SPE. The clean-up for the OCPs and PCBs was carried out by alumina columns to remove lipids. In sediment/soil samples for OCP and PCB analysis, removal of sulphur was performed by copper powder, followed by a fractionation with silica gel. The PCBs and OCPs were analysed by GC-MS/MS, toxaphenes, and PBDEs by GC-MS.

GC-MS/MS conditions for OCP and PCB: Agilent gas chromatography triple quadrupole spectrometer (GC-MS/MS, 7000D) equipped with an electron ionization (EI) source, column: Rxi-5ms (60m x 0.25mm x 0.25um), helix liner, syringe: 50µl.
### Table 1. Countries involved and matrices analysed.

<table>
<thead>
<tr>
<th>Countries by UN Regional Group</th>
<th>Matrices analysed</th>
<th>Number of samples</th>
<th>Reported by country</th>
</tr>
</thead>
<tbody>
<tr>
<td>Africa</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Egypt</td>
<td>Fish (2)</td>
<td>2</td>
<td>No</td>
</tr>
<tr>
<td>Ethiopia</td>
<td>Soil (2), sediment (8)</td>
<td>10</td>
<td>No</td>
</tr>
<tr>
<td>Ghana</td>
<td>Fish (9), shellfish (3), sediment (4)</td>
<td>16</td>
<td>No</td>
</tr>
<tr>
<td>Kenya</td>
<td>Cow milk (2), chicken egg (2), fish (2), soil (1), sediment (2)</td>
<td>9</td>
<td>No</td>
</tr>
<tr>
<td>Morocco</td>
<td>Fish (1), chicken egg (1), sediment (1)</td>
<td>3</td>
<td>No</td>
</tr>
<tr>
<td>Mauritius</td>
<td>Sediment (4), soil (2), fish (2), sugar (1), honey (1), indoor air (1)</td>
<td>11</td>
<td>No</td>
</tr>
<tr>
<td>Nigeria</td>
<td>Sediment (1), soil (1), fish (3), maize (1), beef (1), guinea fowl (1), pulse (1)</td>
<td>9</td>
<td>No</td>
</tr>
<tr>
<td>Senegal</td>
<td>Fish (1), shrimp (1), shellfish (2), chicken egg (1), cow milk (1), butter (1), honey (1), sediment (1)</td>
<td>9</td>
<td>No</td>
</tr>
<tr>
<td>Tanzania</td>
<td>Soil (3)</td>
<td>3</td>
<td>No</td>
</tr>
<tr>
<td>Tunisia</td>
<td>Chicken egg (2), butter (1), olive oil (1)</td>
<td>4</td>
<td>Yes</td>
</tr>
<tr>
<td>Uganda</td>
<td>Sediment (1), soil (2), chicken (1), beef (1), honey (1), chicken egg (1)</td>
<td>7</td>
<td>No</td>
</tr>
<tr>
<td>Zambia</td>
<td>Fish (4), beef (2), maize (1), tomatoes (1)</td>
<td>8</td>
<td>No</td>
</tr>
<tr>
<td>Asia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mongolia</td>
<td>Fish (4), mutton (1), beef (1), chicken (2), pork (1), horse meat (1), chicken egg (4), sea buckthorn (1), flax seeds (1), soil (4)</td>
<td>20</td>
<td>No</td>
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<tr>
<td>Thailand</td>
<td>Sediment (4), fish (4), beef (4), chicken egg (4), duck egg (2),</td>
<td>14</td>
<td>No</td>
</tr>
<tr>
<td>Vietnam</td>
<td>Sediment (8), fish (4), chicken (2)</td>
<td>14</td>
<td>Yes*</td>
</tr>
<tr>
<td>Pacific</td>
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<td></td>
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<tr>
<td>Fiji</td>
<td>Sediment (3)</td>
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<tr>
<td>Samoa</td>
<td>Chicken egg (2)</td>
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</tr>
<tr>
<td>GRULAC**</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Antigua and Barbuda</td>
<td>Fish (2), chicken egg (1), sediment (1), soil (1)</td>
<td>5</td>
<td>No</td>
</tr>
<tr>
<td>Argentina</td>
<td>Fish (1), butter (1), sediment (1)</td>
<td>3</td>
<td>No</td>
</tr>
<tr>
<td>Barbados</td>
<td>Fish (3), chicken egg (2), pork (1), sediment (3), soil (1)</td>
<td>10</td>
<td>No</td>
</tr>
<tr>
<td>Brazil</td>
<td>Sediment (2)</td>
<td>2</td>
<td>No</td>
</tr>
<tr>
<td>Chile</td>
<td>Fish (2), butter (1), chicken egg (2), sediment (1)</td>
<td>6</td>
<td>Yes*</td>
</tr>
<tr>
<td>Colombia</td>
<td>Fish (4), sediment (6)</td>
<td>10</td>
<td>No</td>
</tr>
<tr>
<td>Jamaica</td>
<td>Fish (2), butter (1), chicken egg (1), beef (1), kale (1), oil (1), soil (1), sediment (1)</td>
<td>9</td>
<td>No</td>
</tr>
<tr>
<td>Uruguay</td>
<td>Fish (5), milk powder (1), sediment (2)</td>
<td>8</td>
<td>No</td>
</tr>
</tbody>
</table>

*Only sediment; **GRULAC: Group of Latin-American and Caribbean Countries.
MON-PM1-C1  Results of the UNEP worldwide monitoring of persistent organic pollutants in biota, sediment and food products

GC-MS conditions for toxaphene, PBDE analysis: Agilent gas chromatography-negative chemical ionization-mass spectrometer (GC-NCI MS, 5975). Column (PBDE, HxBB, Toxaphene): CP Sil 8 CB (50m x 0.25mm, 0.25µm), column (BDE209): DB-SHT (15m x 0.25mm, 0.10µm), Ultra Inert Liners, syringe: 10µl.

LC-MS/MS conditions for HBCD analysis: Bruker liquid chromatography tandem mass spectrometer (LC-MS/MS, EVOQ) equipped with an electrospray ionization (ESI) source. Column: Waters Acquity UPLC BEH C18 1.7µm (2.1x150mm).

The GRULAC samples were first freeze-dried or, if dry already, they were directly homogenized and extracted. Soxhlet extraction was performed for all matrices with hexane:DCM (1:1, v:v), except for the determination of PBDEs from sediment and soil for which toluene was used. For PCBs and PBDEs, purification of the extracts was done over multilayer (acid/basic) silica columns followed by fractionation using Florisil and basic alumina columns, respectively. The clean-up of OCPs was carried out directly over a Florisil column to avoid losses of compounds that can degrade in acidic/basic conditions at the silica column. All POPs were analysed by GC-HRMS, using labelled standards. A DB-XLB (60m x 0.25mm, 0.25µm) column was used for the PCB and OCP analysis, and a DB-5ms (40m x 0.18m, 0.18µm) column for PBDE analysis.

Results:
Although 42 countries were invited, samples were only received from 25 countries. While some countries experienced logistic difficulties, such as Brazil (see above) and Samoa, from which several chicken egg samples were lost due to damage during transport, a higher participation degree was expected. This project was meant as a service for the countries. Apparently, even the relatively simple set-up created difficulties in the various laboratories. The reference laboratories invested a lot of effort in communication by email and phone, but in many cases to no avail. In that sense, the project on monitoring POPs in air for which the countries were responsible for the (passive) sampling and sending the samples to the reference laboratories was more successful (de Boer et al., 2023). Even less successful was the ‘mirror’ component of the project. Only Vietnam, Tunisia and Chile were able to analyse the sample they had sent to the reference laboratories also by themselves and report the results on time. The comparison of their results with those obtained by the reference laboratories showed circa 50% of correct results. It should be mentioned that a substantial part of those results were concentrations below the detection limit.

It is not possible to give all data in this short paper. Therefore, we decided to give a representative selection of samples with the most important POP results, and selected only the matrices fish, sediment and butter, as these were analysed most frequently. Many other POPs in other samples were found below the detection limits, and even in this table several POPs are not detectable. All samples (sediment, fish and eggs) from Antigua and Barbuda showed POP concentrations below the detection limits, but in one soil sample total-DDT concentrations of more than 25 µg/kg were found.

### Table 2. Selected representative results, concentrations in fish and butter mg/kg total weight, in sediment in mg/kg dry weight.

<table>
<thead>
<tr>
<th>Country</th>
<th>Matrix</th>
<th>(\Sigma 6)PCB*</th>
<th>(\Sigma) DDT</th>
<th>(\gamma)-HCH</th>
<th>Dieldrin</th>
<th>PentaBDE**</th>
<th>DecaBDE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ghana</td>
<td>Fish</td>
<td>&lt;0.5</td>
<td>0.12-0.63</td>
<td>&lt;0.1</td>
<td>&lt;0.5</td>
<td>&lt;1</td>
<td>&lt;0.4</td>
</tr>
<tr>
<td>Morocco</td>
<td>Fish</td>
<td>0.74</td>
<td>0.79</td>
<td>0.34</td>
<td>&lt;0.9</td>
<td>2.4</td>
<td>0.42</td>
</tr>
<tr>
<td>Senegal</td>
<td>Fish</td>
<td>0.1-1.1</td>
<td>&lt;0.1</td>
<td>0.13</td>
<td>&lt;0.22</td>
<td>&lt;0.1</td>
<td>&lt;0.15</td>
</tr>
<tr>
<td>Tunisia</td>
<td>Fish</td>
<td>134</td>
<td>3.2</td>
<td>0.55</td>
<td>&lt;0.76</td>
<td>0.7</td>
<td>1.5</td>
</tr>
<tr>
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<td>Fish</td>
<td>&lt;0.3</td>
<td>&lt;0.1</td>
<td>&lt;0.11</td>
<td>&lt;0.35</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>Mongolia</td>
<td>Fish</td>
<td>11</td>
<td>0.43-2.1</td>
<td>&lt;0.1-0.22</td>
<td>&lt;0.4</td>
<td>&lt;0.9-1.4</td>
<td>&lt;0.3</td>
</tr>
<tr>
<td>Vietnam</td>
<td>Fish</td>
<td>0.2-0.7</td>
<td>&lt;0.3-0.93</td>
<td>&lt;0.4</td>
<td>&lt;1.0</td>
<td>&lt;0.7</td>
<td>&lt;0.29</td>
</tr>
<tr>
<td>Argentina</td>
<td>Fish</td>
<td>72</td>
<td>18</td>
<td>0.23</td>
<td>na</td>
<td>7.1</td>
<td>na</td>
</tr>
<tr>
<td>Chile</td>
<td>Fish</td>
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<td>1.0-1.1</td>
<td>&lt;0.06</td>
<td>na</td>
<td>0.24</td>
<td>na</td>
</tr>
<tr>
<td>Jamaica</td>
<td>Fish</td>
<td>0.1-0.4</td>
<td>0.05-0.25</td>
<td>&lt;0.05</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>na</td>
</tr>
<tr>
<td>Uruguay</td>
<td>Fish</td>
<td>0.23-0.95</td>
<td>0.09-0.55</td>
<td>&lt;0.03</td>
<td>&lt;0.07</td>
<td>0.07-0.17</td>
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</tr>
<tr>
<td>Ghana</td>
<td>Sediment</td>
<td>&lt;0.2-0.22</td>
<td>&lt;0.2-0.55</td>
<td>&lt;0.2</td>
<td>&lt;0.2</td>
<td>&lt;0.1-0.2</td>
<td>0.05-11</td>
</tr>
</tbody>
</table>
MON-PM1-C1 Results of the UNEP worldwide monitoring of persistent organic pollutants in biota, sediment and food products

Morocco Sediment 0.3 <0.33 12 <0.18 <1.2 0.08
Senegal Sediment 0.3 0.85 <0.17 <0.18 <1 0.50
Vietnam Sediment <0.7-0.93 <0.3-3.1 0.2-46 <0.18 <0.5-1.5 1.4-4.9
Argentina Sediment 20 4.5 0.11 na 3.1 na
Brazil Sediment 73-75 >12 33 na 0.6-1.6 na
Chile Sediment 0.69 <0.2 <0.09 na 0.58 na
Jamaica Sediment 0.27 0.10 na na na na
Uruguay Sediment 0.25 0.27 0.08 <0.16 0.23-0.41 na
Fiji Sediment <0.8 0.02-0.07 <0.19 <0.18 <1.2 0.04-0.05
Senegal Butter <3 <4 <1.0 <1.2 <3 0.17
Tunisia Butter <4 <2.5 <0.9 <1.1 <2.2 0.29
Mongolia Butter na na na na <1 0.16-3.1
Argentina Butter <0.2 10 na na <0.2 na
Chile Butter 0.46 14 na na <0.1 na
Jamaica Butter <0.2 12 na na 0.25 na

na: not analysed. *Sum of PCBs 28, 52, 101, 138, 153 and 180; ** Sum of PBDEs 17, 28, 47, 99, 100, 153, 154, and 183.

Discussion:
As we experienced during a capacity building project that was carried out just prior to this study, and from what was observed from a set of interlaboratory studies (Fiedler et al., 2022, de Boer et al., 2022), the difficulties encountered in the present study, both in sampling and analysis by the countries, further confirm the gap that exists between laboratories in developing countries and those in the western world. A lack of continuity of workflow in the laboratories, a lack of quality of staff, difficulties with investments in instrumentation and ordering of consumables cause that this gap is rather growing than shrinking. In fact, the environmental laboratories in developing countries get much less priority of their governments compared to food laboratories. While this is maybe understandable, the countries all have ratified the Stockholm Convention, and so have accepted responsibility for delivering data to the GMP.

While the ‘mirror’ component of the study was less successful, the dataset of 197 samples is still valuable. It provides information on POP concentrations in various countries, in both the environment and in food stuff. Because the variety of the samples is large, a straight comparison or trend analysis is difficult. Table 2 shows a selection of representative results.

Information about the sampling location is of course essential for a correct interpretation of the results. As an example, the highest levels of all POP analysed in sediment were found in an Argentinian coastal sediment collected at "Río de la Plata". These values were also in agreement with high levels present in a fish from the same site (Table 2). On the contrary, most of the POP concentrations in a sediment from a remote area in Chilean Patagonia were below the detection limits. High concentrations of PCBs and OCPs were also found in a sediment from the Sao Paulo region (Brazil), e.g., PCB 73-75 mg/kg and ∼-HCH 33 mg/kg, and a significant level of total DDT with a $p,p'$-DDT/$p,p'$-DDE ratio around 1. These findings could be related to important agricultural and industrial activities in the sampling area. DDT and their metabolites were also present at high levels in a soil sample from Antigua and Barbuda, and to some extent also in several fish and food samples, particularly in butter, from different GRULAC countries. However, in all these samples the $p,p'$-DDT/$p,p'$-DDE ratio was below 1. It is also remarkable that total-DDT concentrations are generally higher in Latin America than in Africa, possibly due to application in a later stage. A high PCB concentration (134 mg/kg) was found in a Tunisian fish but was not reflected in the Tunisian butter sample. One Mongolian soil sample had a g-HCH concentration of 29 mg/kg, and a second soil sample 2 mg/kg g-HCH, while two other soil samples were substantially lower in g-HCH.

Interpretation of results should be done with care, as the emphasis in this study was on developing countries. Consequently, results from colder areas, especially on the northern hemisphere, where POP concentrations might be higher, are lacking here.
MON-PM1-C1  Results of the UNEP worldwide monitoring of persistent organic pollutants in biota, sediment and food products

Conclusions:
Valuable information on the worldwide distribution of POPs in fish, sediments, soil and food items was obtained. Some of the OCPs, such as heptachlor epoxide, oxychlordane, mirex and toxaphene were non-detectable or only present in very low concentrations. However, DDT/DDE and PCBs, as well as PBDEs are still being found, sometimes at substantial levels. This again shows how much, once produced in high volumes, these persistent halogenated substances threaten several generations.

References:
Introduction:
Recently, organophosphorus flame retardants (OPFRs) have been introduced, among other flame retardants, as replacements for the discontinued polybrominated diphenyl ethers (PBDEs) because of their excellent thermal and hydrolytic stabilities and ease of synthesis. However, more environmental attention is beginning to identify organophosphate tri-esters with some environmental problems. Because OPFRs are additively bonded to most polymeric materials, they can easily be released via volatilization and leaching. Over time, OPFRs treated products dumped into landfills may degrade, resulting in the gradual release of contaminants, particularly during periods of intense precipitation. The resultant leachate, is a complex cocktail that may contain elevated concentrations of OPFRs and other contaminants.

In recent times, great attention has been given to the use of green solvents to extract organic contaminants in environmental samples. This is simply because of their very low toxicity and volatility compared to the widely used organic solvents. It is in this light that ionic solvents and recently deep eutectic solvents (DES) are now widely used in various extractions. DES are gradually replacing ionic liquids because of their unique properties and environmental friendliness. Information on extraction of OPFR compounds in aqueous media, especially in sediment using hydrophilic DES is still very scarce. DES are inexpensive to prepare, thermally stable, and are biodegradable, and their application in extraction exercises is a boost to the quest for green solvents. In this study, hydrophilic DES were used to extract OPFRs from landfill sediment.

Materials and Methods:
Stable isotope labelled, unlabeled and CRM standards were obtained from the first worldwide interlaboratory study on OPFRs. All solvents, dichloromethane, ethyl acetate, hexane, methanol and formic acid were HPLC grade. All glassware was washed with detergent, rinsed with Milli-Q water, acetone, hexane and finally oven dried. Prior to use, the clean glassware was rinsed with the extraction solvent. Ultrapure water was dispensed from Labostar ultrapure. Analytical grades of choline chloride (C\textsubscript{5}H\textsubscript{14}ClNO, 99.0 %), urea ((NH\textsubscript{2})\textsubscript{2}CO, 98 %), oxalic acid dihydrate (C\textsubscript{2}H\textsubscript{2}O\textsubscript{4}·2H\textsubscript{2}O, 98 %), silicon oil standard and hexadeuterodimethyl sulfoxide (DMSO-d\textsubscript{6}) were used in the synthesis of DES.

Deep eutectic solvents were prepared by heating the HBA (hydrogen bond acceptor):HBD (hydrogen bond donor) mixtures at 80 °C for Choline/Urea (1:2) and 90 °C for Choline/Oxalic acid (1:1) with constant stirring until a homogeneous liquid was formed. DES was characterised for densities, refractive indices, melting points and viscosities and thereafter used in solid-liquid extraction of OPFRs. A procedural blank was analysed every ten samples to check for laboratory contamination. Spiking method was used in the quality assurance process in addition with the CRM. Recoveries of the internal standards (IS) spiked formed. DES was characterized for densities, refractive indices, melting points and viscosities and thereafter used in solid-liquid extraction of OPFRs. A procedural blank was analysed every ten samples to check for laboratory contamination. Spiking method was used in the quality assurance process in addition with the CRM. Recoveries of the internal standards (IS) spiked were obtained from the LC-MS/MS for all targeted OPFR calibrations. LODs and LOQs were calculated based on the signal/noise ratio for a standard of known concentration. The LOD was three times the signal to noise ratio (S/N 3:1) and for LOQ it was ten times the signal to noise ratio (S/N 10:1) of the lowest calibration level.

Chromatographic separation of OPFRs was achieved on an InertSustain C18 column (3 µm particle size, 2.1 × 150 mm). The column temperature was set to 40 °C. The mobile phase consisted of a mixture of 0.1% formic acid (FA) in water (solvent A) and MeOH (solvent B). Binary gradient elution of 0.30 mL/min with 80 % pump B flow was used. starting with a mixture of 50 % solvent A for 0.01 min; then from 50 % to 20 % (solvent A) for 3 min; 12 min, 20 %; 15 min, 20 %; 17 min, 50 %; and 20 min, 50 %. A sample aliquot of 10 µL was injected throughout the analysis. Standards and the test samples were subjected to a run-time of
MON-PM1-C2  Green Deep Eutectic Solvent Extraction of Phosphorus Flame Retardants in Landfill Sediment

20 min. All the target compounds in this study were analysed in negative ESI mode. The source heating-block was maintained at 400 °C, while the desolvation temperature of 250 °C was employed and a drying gas flow of 15 L/min. Nitrogen was used as the drying and nebulising gas (3 L/min), while the collision-induced dissociation (CID) gas was argon and was maintained at 230 kPa. The resulting fragment ions were monitored in multiple reaction monitoring (MRM) mode with a dwell time of 100 ms.

Results:
LOD and LOQs ranged from 0.001–0.01 ng/g and 0.08 – 0.12 ng/g dw respectively. Fourteen different OPFR compounds were targeted in this study, three chlorinated (TCEP, TCPP and TDCPP), five alkylated (TEP, TPrP, TBEP, EHDP and TEHP) and six arylated (TTP, TOTP, TMTP, T35DMPP, T21PPP and TPTP). Figures 1-3 show box plots of chlorinated, alkylated and arylated OPFRs concentrations observed in this study. TCEP and TCPP (Figure 1) minimum concentrations ranged from <LOQ (TCPP, Johannesburg landfill sites)–189 ng/g dw (TCPP, Hatherly), with maximum concentration range between 48.0 ng/g dw (TCPP, Goudkoppies)–8.30 x10^3 ng/g dw (TCPP, Onderstepoort). The median concentrations ranged from <LOQ (TCPP; Goudkoppies and Marie Louis)–4.04 x10^3 ng/g dw (TCPP, Onderstepoort) in all the landfill sites.

Figure 1: Concentrations of chlorinated OPFRs in landfill sediment from Tshwane (HAT=Hatherly, SOSH=Soshanguve, OND=Onderstepoort, GAR=Garankuwa) and Johannesburg (ENN=Ennerdale, GOU=Goudkoppies, MAR=Marie Louis, ROB=Robinson Deep).

Alkylated OPFR compounds (Figure 2) had minimum concentrations that ranged from <LOQ for all compounds in Johannesburg landfill sites except EHDP; however, in Tshwane, it was TBEP and TEP in all the landfill sites, as well as TEHP and EHDP in Onderstepoort and these were up to 266 ng/g dw (TPrP, Garankuwa). TPrP exhibited maximum OPFRs concentrations of up to 669 ng/g dw in Onderstepoort landfill site. For the median OPFRs concentrations, TEP was <LOQ in all Tshwane and Johannesburg landfill sites. However, 395 ng/g dw, was recorded as the highest level for TPrP in Onderstepoort landfill site.
For the arylated compounds (Figure 3), minimum concentrations of <LOQ were observed in all the sites for TOTP, TMTP and T35DMPP, including TPTP and T21PP in all Johannesburg landfill sites. TPP was detected in all the landfill sites at minimum concentrations of 50.8 ng/g dw. TPP was observed in all targeted landfill sites, and it exhibited maximum concentrations of 213 ng/g dw in Garankuwa landfill site. However, all other OPFRs were <LOQ in Onderstepoort except TPP. Other compounds such as TMTP in Goudkoppies and Robinson Deep, TPTP and T35DMPP in Marie Louis, as well as T35DMPP in Robinson Deep were also <LOQ. On the other hand, the highest median concentration of 119 ng/g dw was exhibited by TMTP in Ennerdale landfill site; whereas TOTP and T21PP were <LOQ in all Johannesburg landfill sites.
Discussion:
Due to scanty reports on OPFRs in landfill sediment, studies on e-waste soils were used to compare the results obtained in the present study. The median concentrations of <LOQ–4.04 x10^3 ng/g dw that were observed in this study are within the range, nd–4.99 x10^3 ng/g dw, reported in landfill soil samples in Brazil 2. A study of soil samples by Wang et al 7 in China from a multi-waste recycling area reported OPFRs concentration that ranged from 37.7–2.10 x10^3 ng/g dw. Matsukami et al 8 reported OPFRs concentrations in e-waste soils from Vietnam which ranged between 41.0–280 ng/g dw. These values are lower than the values observed in the present study. The observed difference could be due to the multiple OPFR sources from municipal landfill waste as opposed to the selective sources (electronic) of OPFRs from e-waste recycling sites.

Conclusion:
The presence of 13 out of 14 targeted OPFRs from landfill sediment was confirmed in this study. Tshwane and Johannesburg landfill sites were dominated by chlorinated and alkylated OPFRs respectively. The median OPFR concentrations (<LOQ–4.04 x10^3 ng/g dw) from this study were comparable to OPFRs in landfill soil in Brazil, however, they were higher than the reported levels from e-waste recycling soils in China and Vietnam. The high TCEP and TCPP levels observed in this study are of concern due to their possible adverse health effects.

Acknowledgements:
This work was supported by Tshwane University of Technology (TUT) during Dr (Mrs). IV Sibiya’s PhD studies. The authors are also indebted to Prof Adriaan Covaic for the provision of standards and hosting Dr Sibiya under the EU programme, INTERWASTE headed by Prof Stuart Harrad.

References:
9. https://doi.org/10.1016/j.chemosphere.2016.09.147
MON-PM1-C3 POPs in Plastic Products and Chicken Eggs from Kenya in the Light of the Basel and Stockholm Conventions

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Introduction:
Developing countries, including countries in Africa, suffer from the health and environmental impacts of toxic chemicals and wastes more than developed countries. This is, in part the result of loopholes in international legislation and exploitation of those loopholes by large corporations and countries that export e-waste and end-of-life vehicles (ELVs) to Africa. Due to the lack of sound waste management, imported materials, frequently containing dangerous chemicals, are often managed poorly or in a hazardous manner including weak recycling, dumping or open burning. Burning such wastes generates new, even more toxic chemicals, such as chlorinated and brominated dioxins and polyaromatic hydrocarbons.4

This study aims at determining whether persistent organic pollutants (POPs) find their way into consumer products made of recycled plastics (products that tend to come from recycled e-waste plastics and plastics from ELVs) and human food in Kenya. This is because of weak waste management practices and their impact on food sources in areas around waste disposal sites. The findings in this study were compared to the previous research and the relevant international regulations. More details about the research including description of localities, samples and analytical methods are included in a broader report by IPEN, Arniko and CEJAD4.

Materials and Methods:
Free-range chicken eggs were sampled in the vicinity of potential POPs pollution hot spots. The hot spots are either sites where the waste containing POPs (e.g. used electronic and electrical equipment, car wrecks, or various plastic waste) is handled or facilities that burn wastes containing halogenated compounds (mainly plastic). For this study, we chose four hot spots in Kenya for collecting free-range chicken egg samples:

1. Nairobi – Dandora, which has a large dumpsite where there is often open burning of plastic waste;
2. Nairobi – Ngara market, which is known as the e-waste dismantling site inside the city;
3. Nairobi – Mirema, where the so-called “community cooker” uses plastic waste as fuel for cooking; and
4. Nanyuki, a dumpsite with open burning and e-waste disposal.

Free-range chicken eggs collected at each location were analyzed as a pooled sample of 5 – 8 eggs (see Table 1) to get more representative results15,6. We also purchased eggs from a supermarket in Nairobi and sent them for analysis, for use as reference sample. The eggs were hard boiled before being transported to laboratories in Europe.

Sample preparation is described in previous studies7,8. The eggs were analyzed for the following POPs: polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs), polychlorinated biphenyls (PCBs), polybrominated dibenzo-p-dioxins and dibenzofurans (PBDD/Fs), hexachlorobenzene (HCB), hexachlorobutadiene (HCBD), polychlorinated naphthalenes (PCNs), short-chain chlorinated paraffins (SCCPs), 3 isomers of hexachlorocyclohexane (HCH) 6 isomers of dichlorodiphenyldichloroethane (DDT), 3 isomers of hexabromocyclododecane (HBCD), polybrominated diphenyl ethers (PBDEs), six novel brominated flame retardants (nBFRs), and per- and polyfluoroalkyl substances (PFASs). Chemicals, their properties, and potential health effects are described in Annex 1 to a broader study or elsewhere9,10. All egg samples were analyzed for POPs in certified laboratories. PCDD/Fs, and dioxin-like PCBs (dl PCBs) were analyzed at the laboratory of State Veterinary Institute Prague. PBDD/Fs were analyzed at the laboratory MAS based in Muenster, Germany; all other POPs were analyzed at the University of Chemistry and Technology in Prague, Czech Republic. Chemical analyses for POPs in eggs or similar matrices in these laboratories were described in previous studies10,11. Results of the analyses are summarized in Table 1.

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1PCB congeners representing both groups of intentionally (represented by 6 or 7 indicator congeners) and unintentionally (expressed by 12 dioxin-like PCB congeners) produced PCBs were analyzed.
2Alfa-, beta- and gamma-isomers were analyzed. The gamma isomer is known as lindane.
316 congeners representing commercial mixtures of Penta-, Octa-, and DecaBDE were analyzed.
4This group of chemicals was represented in our analyses by the following chemicals: 1,2-bis(2,4,6-trimethylpheny)-1-indane (OBIND), 2,3,4,5-tetabromobenzylbenzene (TBBE), pentabromobenzene (PBEB), hexabromobenzene (HBB), octabromodiphenyl ethane (OBDE), decabromodiphenyl ethane (DBDE), hexabromobenzene (HBB), hexabromobenzene (HBB), hexabromocyclododecane (HBCD), 2,3,4,5,6-pentabromodiphenylmethane (PBDM), and pentabromodibenzofuran (PBF).
5PFASs and/or their groups were analyzed, including linear (L-PFOS) as well as branched (br-PFOS) perfluorooctane sulfonate isomers, perfluoro-n-octanoic acid (PFOA) and perfluorooxyhexane sulfonic acid (PFHxS).
Ninety-six products, including children’s toys, hair accessories, kitchen utensils, and office supplies, were purchased from markets in Kenya. Since the products were made of recycled plastics.

As X-ray fluorescence is a useful technique for determining the presence of PBDEs in plastics, all samples were screened using a handheld NITON XL3t 800XRF analyzer to guide the selection of samples for further laboratory analysis.

The method for collecting the samples was described in the previous study. Following the screening, 18 products with elevated levels of bromine and antimony – 7 hair accessories, 3 kitchen utensils, 4 office utensils and 4 toys – were selected for laboratory analysis at the University of Chemistry and Technology based in Prague, Czech Republic. Groups of PBDEs, HBCD and nBFRs, and tetrabromobishpenol A (TBBPA) were analyzed in these products. The used analytical methods are explained in previous studies. The results of analyses for BFR in plastic products are summarized in Table 2.

In addition to the above mentioned BFRs, one sample, a toy car, was also analyzed for brominated dioxins for dioxin-activity by DRhumanCALUX using the analytical methods described in the previous study by Budin et al. The results were published by Grechko et al. (2022). A daily dietary intake was calculated for PCDD/Fs + dl-PCBs, PBDD/Fs, and PFOS. The results of the calculations were compared with the tolerable daily intake (TDI) established by different regulatory authorities (EFSA and WHO). The calculations were made by using measured levels of certain chemicals per gram of weight of the fresh egg and a calculation of a daily intake of 1/10 of an egg per day (3.6 grams of egg weight) which is an average consumption of eggs in Kenya. The average body weight of 61 kg for Africa was taken from information about average human body weight in different parts of the world. There is no TDI for PBDD/Fs established yet. For this study, a summary of brominated, chlorinated dioxins and dioxin-like PCBs was compared to the TDI for PCDD/Fs + dl PCBs.

### Results:
The results of analyses of eggs and consumer products are summarized in Tables 1 and 2.

<table>
<thead>
<tr>
<th>Locality</th>
<th>Sample ID</th>
<th>Nairobi - Mirema</th>
<th>Nairobi - Ngara market (e-waste)</th>
<th>Nairobi – Dandora</th>
<th>Nanyuki</th>
<th>Nairobi superm.</th>
<th>EU limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample ID</td>
<td></td>
<td>KE_001</td>
<td>KE_002</td>
<td>KE-EG-NG_001</td>
<td>KE-EG-D002</td>
<td>KE-EG-NY_003</td>
<td>KE_SUP</td>
</tr>
<tr>
<td>Number of eggs per sample</td>
<td>5</td>
<td>6</td>
<td>6</td>
<td>8</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Fat content (%)</td>
<td>14</td>
<td>16</td>
<td>19</td>
<td>19</td>
<td>17</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>PCDD/Fs (pg TEQ/g fat)</td>
<td>12</td>
<td>18</td>
<td>12</td>
<td>19</td>
<td>5.0</td>
<td>0.22</td>
<td>2.5</td>
</tr>
<tr>
<td>dl PCBs (pg TEQ/g fat)</td>
<td>2.1</td>
<td>502</td>
<td>555</td>
<td>7.0</td>
<td>1.7</td>
<td>0.05</td>
<td>-</td>
</tr>
<tr>
<td>PCDD/F + dl PCBs (pg TEQ/g fat)</td>
<td>14</td>
<td>520</td>
<td>567</td>
<td>26</td>
<td>6.7</td>
<td>0.27</td>
<td>5.0</td>
</tr>
<tr>
<td>PBDD/Fs (pg TEQ/g fat)</td>
<td>NA</td>
<td>8.5</td>
<td>0.61</td>
<td>12</td>
<td>2.4</td>
<td>0.043</td>
<td>-</td>
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<tr>
<td>HCB (ng/g fat)</td>
<td>0.71</td>
<td>1.4</td>
<td>2.4</td>
<td>37</td>
<td>0.60</td>
<td>&lt;0.1</td>
<td>-</td>
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<tr>
<td>PeCB (ng/g fat)</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>1.36</td>
<td>34</td>
<td>0.15</td>
<td>&lt;0.1</td>
<td>-</td>
</tr>
<tr>
<td>HCBOD (ng/g fat)</td>
<td>1.50</td>
<td>3.41</td>
<td>&lt;0.10</td>
<td>1.30</td>
<td>&lt;0.10</td>
<td>0.71</td>
<td>-</td>
</tr>
<tr>
<td>7 PCB (ng/g fat)**</td>
<td>0.73</td>
<td>3,137</td>
<td>2,103</td>
<td>21</td>
<td>6.12</td>
<td>11</td>
<td>-</td>
</tr>
<tr>
<td>6 PCB (ng/g fat)*</td>
<td>0.73</td>
<td>2,235</td>
<td>1,282</td>
<td>20</td>
<td>4.46</td>
<td>8.10</td>
<td>40.0</td>
</tr>
<tr>
<td>13 PCN (ng/g fat)</td>
<td>NA</td>
<td>NA</td>
<td>&lt;0.20</td>
<td>&lt;0.20</td>
<td>&lt;0.20</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>SCCPs (ng/g fat)</td>
<td>102</td>
<td>224</td>
<td>65</td>
<td>383</td>
<td>&lt;50.0</td>
<td>1,441</td>
<td>-</td>
</tr>
<tr>
<td>sum HBCD (ng/g fat)</td>
<td>287</td>
<td>&lt;4.2</td>
<td>&lt;4.2</td>
<td>&lt;4.2</td>
<td>&lt;4.2</td>
<td>&lt;4.2</td>
<td>-</td>
</tr>
<tr>
<td>sum of PBDEs (ng/g fat)</td>
<td>24</td>
<td>75</td>
<td>31</td>
<td>639</td>
<td>85</td>
<td>&lt; LOQ</td>
<td>-</td>
</tr>
<tr>
<td>sum of nBFRs (ng/g fat)</td>
<td>3.6</td>
<td>10</td>
<td>18</td>
<td>31</td>
<td>&lt; LOQ</td>
<td>&lt; LOQ</td>
<td>-</td>
</tr>
<tr>
<td>sum of PFASs (ng/g of fresh weight)</td>
<td>1.46</td>
<td>NA</td>
<td>6.65</td>
<td>1.97</td>
<td>1.25</td>
<td>0.34</td>
<td>-</td>
</tr>
</tbody>
</table>

Total content of toxic chemical(-s) in one egg (36 g)
AFTERNOON BREAKOUT SESSIONS I  
MONDAY 11 SEPTEMBER 2023

13:30 - 15:10

POPs in Developing Countries

K. Pozo & B. Gevaño

MON-PM1-C3  POPs in Plastic Products and Chicken Eggs from Kenya in the Light of the Basel and Stockholm Conventions

<table>
<thead>
<tr>
<th></th>
<th>PCDD/F + dl PCBs (pg TEQ/g fw)</th>
<th>PBDD/Fs (pg TEQ/g fw)</th>
<th>PFOS (ng/g fw)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>69</td>
<td>2993</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>3881</td>
<td>176</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>41</td>
<td>0.98</td>
<td>15</td>
</tr>
</tbody>
</table>

Intake per kg of body weight for an adult person (61 kg on average) when eating 1/10 of an egg (3.6 g)

<table>
<thead>
<tr>
<th></th>
<th>PCDD/F + dl PCBs (pg TEQ/kg bw)</th>
<th>PBDD/Fs (pg TEQ/kg bw)</th>
<th>PFOS (ng/kg bw)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.11</td>
<td>0.08</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>4.91</td>
<td>0.01</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>6.36</td>
<td>0.13</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>0.29</td>
<td>0.13</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>0.07</td>
<td>0.02</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Exceedance of total daily tolerable intake when eating 1/10 of an egg (3.6 g) per day

<table>
<thead>
<tr>
<th></th>
<th>PCDD/F + dl PCBs (EFSA 2018) x</th>
<th>PBDD/Fs (EFSA 2018)</th>
<th>PFOS (EFSA 2018)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.45</td>
<td>0.08</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>19.62</td>
<td>0.01</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>25.45</td>
<td>0.13</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>1.15</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>0.27</td>
<td>0.0003</td>
<td>0.01</td>
</tr>
</tbody>
</table>

NA = not applicable; * sum of PCB28, PCB52, PCB101, PCB138, PCB153, and PCB180; ** sum of PCB28, PCB52, PCB101, PCB138, PCB153, PCB180 and PCB118; ^ 0.25 pg TEQ/kg bw; ^ 2 pg TEQ/kg bw; ^ 6 ng/kg bw per week = approximately 0.86 ng/kg bw per day.

Table 2: Overview of BFRs in consumer products from Kenya, in mg/kg.

<table>
<thead>
<tr>
<th>No</th>
<th>Sample ID</th>
<th>Group</th>
<th>Penta BDE</th>
<th>Octa BDE</th>
<th>Deca BDE</th>
<th>Sum of PBDEs</th>
<th>Sum of HBCD</th>
<th>TBBPA</th>
<th>Sum of nBFRs</th>
<th>Total BFRs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>KEN-H-4</td>
<td>H</td>
<td>&lt;LOQ</td>
<td>46</td>
<td>69</td>
<td>115</td>
<td>0.2</td>
<td>33</td>
<td>154</td>
<td>302</td>
</tr>
<tr>
<td>2</td>
<td>KEN-H-6</td>
<td>H</td>
<td>&lt;LOQ</td>
<td>41</td>
<td>64</td>
<td>106</td>
<td>1.1</td>
<td>50</td>
<td>80</td>
<td>236</td>
</tr>
<tr>
<td>3</td>
<td>KEN-H-7</td>
<td>H</td>
<td>&lt;LOQ</td>
<td>60</td>
<td>85</td>
<td>145</td>
<td>&lt;LOQ</td>
<td>48</td>
<td>82</td>
<td>276</td>
</tr>
<tr>
<td>4</td>
<td>KE-H-03</td>
<td>H</td>
<td>0.2</td>
<td>79</td>
<td>144</td>
<td>223</td>
<td>&lt;LOQ</td>
<td>75</td>
<td>95</td>
<td>393</td>
</tr>
<tr>
<td>5</td>
<td>KE-H-16</td>
<td>H</td>
<td>0.2</td>
<td>149</td>
<td>130</td>
<td>279</td>
<td>0.9</td>
<td>458</td>
<td>412</td>
<td>1,149</td>
</tr>
<tr>
<td>6</td>
<td>KE-H-12</td>
<td>H</td>
<td>0.2</td>
<td>101</td>
<td>57</td>
<td>158</td>
<td>0.4</td>
<td>980</td>
<td>208</td>
<td>1,347</td>
</tr>
<tr>
<td>7</td>
<td>KE-H-02</td>
<td>H</td>
<td>0.005</td>
<td>23</td>
<td>72</td>
<td>95</td>
<td>0.3</td>
<td>24</td>
<td>29</td>
<td>149</td>
</tr>
<tr>
<td>8</td>
<td>KE-K-10</td>
<td>K</td>
<td>0.1</td>
<td>16</td>
<td>52</td>
<td>65</td>
<td>&lt;LOQ</td>
<td>0.7</td>
<td>17</td>
<td>83</td>
</tr>
<tr>
<td>9</td>
<td>KE-K-25</td>
<td>K</td>
<td>0.03</td>
<td>1.8</td>
<td>6</td>
<td>8</td>
<td>&lt;LOQ</td>
<td>0.1</td>
<td>0.9</td>
<td>9</td>
</tr>
<tr>
<td>10</td>
<td>KE-K-15</td>
<td>K</td>
<td>0.1</td>
<td>36</td>
<td>80</td>
<td>116</td>
<td>0.04</td>
<td>63</td>
<td>90</td>
<td>269</td>
</tr>
<tr>
<td>11</td>
<td>KEN-O-5</td>
<td>O</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>0.02</td>
<td>&lt;LOQ</td>
<td>0.02</td>
<td>&lt;LOQ</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>12</td>
<td>KE-O-17</td>
<td>O</td>
<td>0.006</td>
<td>5</td>
<td>78</td>
<td>83</td>
<td>0.1</td>
<td>16</td>
<td>22</td>
<td>122</td>
</tr>
<tr>
<td>13</td>
<td>KE-O-15</td>
<td>O</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>0.2</td>
<td>&lt;LOQ</td>
<td>0.2</td>
<td>&lt;LOQ</td>
<td>0.04</td>
<td>0.3</td>
</tr>
<tr>
<td>14</td>
<td>KEN-O-1</td>
<td>O</td>
<td>&lt;LOQ</td>
<td>9</td>
<td>81</td>
<td>90</td>
<td>0.1</td>
<td>12</td>
<td>10</td>
<td>112</td>
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<tr>
<td>15</td>
<td>KEN-T-6</td>
<td>T</td>
<td>&lt;LOQ</td>
<td>26</td>
<td>243</td>
<td>269</td>
<td>&lt;LOQ</td>
<td>0.5</td>
<td>48</td>
<td>318</td>
</tr>
<tr>
<td>16</td>
<td>KE-T-16</td>
<td>T</td>
<td>0.05</td>
<td>12</td>
<td>119</td>
<td>132</td>
<td>0.05</td>
<td>18</td>
<td>30</td>
<td>180</td>
</tr>
<tr>
<td>17</td>
<td>KE-T-01</td>
<td>T</td>
<td>&lt;LOQ</td>
<td>17</td>
<td>137</td>
<td>153</td>
<td>&lt;LOQ</td>
<td>10</td>
<td>19</td>
<td>182</td>
</tr>
<tr>
<td>18</td>
<td>KE-T-04</td>
<td>T</td>
<td>&lt;LOQ</td>
<td>1.9</td>
<td>7</td>
<td>9</td>
<td>0.1</td>
<td>1.0</td>
<td>6</td>
<td>16</td>
</tr>
</tbody>
</table>

Discussion:

Free-range chicken eggs samples: In most cases, the analyzed POPs levels in the eggs from the selected hot spots in Kenya exceeded by many times the levels measured in the reference sample from a supermarket in Nairobi.

The levels of PCDD/Fs in free-range egg samples in this study were two to eight times higher than the EU regulatory limit of 2.5 pg TEQ/g fat. The sum of PCDD/Fs + dl PCBs was 100 and 111 times, respectively, above the EU regulatory limit of 5 pg TEQ/g fat in two pooled egg samples from the Ngara market. Unintentionally produced POPs such as PCDD/Fs, PBDD/Fs, HCB and PeCB were found in high levels in free-range eggs from the Dandora dumpsite, which may be a result of open burning.
A very high level of 639 ng/g fat of PBDEs was found in the pooled egg sample from the Dandora dumpsite. Ngara market and Dandora dumpsite are comparable with concentrations in eggs from Bagong Silang, an e-waste site in the Philippines. The contamination was also high in one egg sample from the Ngara market. Levels of PBDD/Fs measured in eggs from the Ngara market and Dandora dumpsite are comparable with concentrations in eggs from Bagong Silang, an e-waste site in the Philippines. A very high level of 639 ng/g fat of PBDEs was found in the pooled egg sample from the Dandora dumpsite. This is 22 times higher than the 29 ng/g fat found in pooled egg samples from the Dandora dumpsite in 2004. This result, combined with a high level of PCBs in the sample from the Dandora dumpsite, suggests that plastics from electronics and/or car wrecks are the most likely source of this contamination at Dandora dumpsite. PBDD/Fs can be directly released from such plastics as they are unintentional contaminants of PBDE and nBFR additives in plastics and/or can be formed by burning waste containing BFRs. Based on the analyses in Table 1 the average per capita consumption of eggs at Ngara market, Nairobi (36 eggs per year in Kenya), would exceed the TDI for PCDD/Fs + dl PCBs by 5 to 6 times. In addition, we can also say that by eating just one egg from the Ngara market, one person would surpass the TDI set by EFSA for dioxins and dioxin-like compounds for almost 200 to more than 250 days. The toxic burden caused by POPs in the food chain is even higher for children because of their low body weight and vulnerability.

Samples of consumer plastic products: The laboratory analysis of 18 samples of consumer products (see Table 2) made of recycled plastic purchased in Kenya revealed that 14 out of the 18 samples exceeded the EU regulatory limit of 243 ppm. Results of consumer products showed the presence of DecaBDE in seventeen products at concentrations ranging from 0.1 ppm to 980 ppm. Previous studies compared results of analyses for BFRs in plastic consumer products from Kenya with results from ten other African and Arabic countries. Samples from Kenya show the highest levels of TBBPA and belong to those with higher levels of nBFRs.

Results: In this study agree with previous studies of consumer products from African and Arab countries. Our study finds similar findings: 1) E-waste and ELVs plastic containing high levels of toxic flame retardants should be banned from entering the recycling chain. Also, the loophole allowing exports of non-functional electronics under the guise of repair in the Basel Convention’s Technical Guidelines needs to be removed and stricter standards for the definition of hazardous wastes must be established under both the Basel and Stockholm Conventions. 2) To eliminate human exposure to PBDEs and related harmful chemicals such as brominated dioxins (PBDD/Fs) in products and wastes, strict limit values must be established. Low POPs Content Levels (LPCLs) for waste should be established at a level of 50 ppm as proposed by the African region and accompanied with setting an unintentional trace contamination (UTC) level at 10 ppm. 3) Wastes containing high levels of POPs can be treated by non-combustion technologies, which destroy POPs and do not generate new ones.

Conclusions: In agreement with previous studies from other countries, the present study shows that children’s toys, hair accessories, office supplies, and kitchen utensils found on the Kenyan market are affected by the unregulated recycling of e-waste plastics, which carry persistent toxic pollutants into new products. Leakage and emissions of POP additives from waste are a source of contamination of free-range chicken eggs with BFRs and PFASs in the vicinity of dumpsites and/or community cookers using plastic waste as fuel. Burning plastic waste containing chlorinated and brominated additives generates unintentionally produced POPs (U-POPs) such as HCB, PeCB, PCDD/Fs, PBDD/Fs and dl PCBs. All forms of burning plastic waste, including their use as fuel, should be banned as this releases POPs into the environment. Wastes containing high levels of POPs can be treated by non-combustion technologies, which destroy POPs and do not generate new POPs. The results of this study also highlight that the new global Plastics Treaty should focus on the chemical content of plastics. Other tools that should be established to address issue of contamination of consumer products with POPs such as stricter Low POPs Content level and better control of e-waste transboundary movement were specified in this and previous studies. The elevated levels of PBDEs and nBFRs in some consumer products reported in this study and the known and unknown adverse effects of these chemicals
AFTERNOON BREAKOUT SESSIONS I  MONDAY 11 SEPTEMBER 2023

13:30 - 15:10

POPs in Developing Countries
K.Pozo & B.Gevao

MON-PM1-C3  POPs in Plastic Products and Chicken Eggs from Kenya in the Light of the Basel and Stockholm Conventions

require a class-based approach to the restriction of BFRs.

Acknowledgments:

IPEN, Arnika and CEJAD gratefully acknowledge the financial support provided by the Government of Sweden, the Sigrid Rausing Trust, the Global Greengrants Fund, and other donors that made the production of this document possible.

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MON-PM1-C3  POPs in Plastic Products and Chicken Eggs from Kenya in the Light of the Basel and Stockholm Conventions

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MON-PM1-C4 Characteristics of Polychlorinated dibenzo-p-dioxins and Polychlorinated dibenzofurans in fly ash samples collected at Secondary Aluminum Smelter and Waste to Energy Incinerators in Vietnam

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2Graduate University of Science and Technology, Viet Nam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay Ha Noi 100000, Viet Nam.
3Faculty of Chemistry, University of Science, Vietnam National University, 19 Le Thanh Tong, Hoan Kiem, Hanoi 100000, Viet Nam.

1. Introduction:
Polychlorinated dibenzo-p-dioxin and dibenzofurans (PCDD/Fs) are the most toxic organic compounds, which were listed in the Stockholm convention annex6. PCDD/Fs were formed from burning processes as waste incinerators; metallurgy processes... in stack gas and fly ash1,2,7,8. 17 isomers of PCDD/Fs have four chlorine atoms at 2,3,7,8 places on carbon rings, which are considered most toxic compounds and 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) exhibited the highest toxic compound in dioxin/furans group (Toxic Equivalence Factor = 1)6. Waste-to-Energy incinerators played noticeable roles in dioxin inventory in the USA5,7,8. Vietnam national regulations only mentioned seventeen 2,3,7,8-substituted isomers while non-2,3,7,8 substituted congeners also emitted to environment. The study focuses on characteristic of seventeen toxic 2,3,7,8-substituted PCDD/Fs isomers, and total of four other non-2,3,7,8-substituted TCDD congeners in fly ash samples collected at several secondary aluminum (SAI) smelters and waste-to-energy (WtE) incinerators located in northern Vietnam.

2. Materials and Methods:
2.1. Sample preparation
Five fly ash samples were collected at stack gas treatment systems of Waste-to-Energy (WtE) incinerators (n=3) and secondary aluminum smelter incinerators (n=2) located in northern Vietnam. Samples after collection were pre-treated with 1M HCl acid (a g sample for (8 x a) mL of 1M HCl acid), in 2h at the laboratory. The HCl solution removal was performed by centrifugation at 6000 rpm, aspirating the top layer of HCl solution, then washing with 40 mL of deionized water (this process was repeated three times). After that sample was dried overnight in an oven at 30°C. C 13 label solution from Isotope Cambridge Laboratory as surrogate Standard solution was spiked before extracting on Accelerated Solvent Extraction (Thermo ASE-350 system) and extraction by toluene in 1.5h. Multilayer silica and carbon columns were used to clean-up and then concentrated by nitrogen gas stream (99.99% purity) to 20uL. C13 internal standard solution was also spiked to samples.

2.2. PCDD/Fs analysis
High resolution gas chromatography (HRGC) Trace 1310 coupled with high resolution mass spectrometry (HRMS) DFS (Thermo, USA) was used to identify and quantified for seventeen isomers of 2,3,7,8-PCDD/Fs and 21 isomers of non-2,3,7,8 tetrachloro dibenzo-p-dioxins. 2,3,7,8-PCDD/Fs were analyzed by Isotope dilution technique. High resolution mass spectrometer equipment was operated in the selected ion monitoring (SIR) mode at resolution of > 10.000 and based on using of TG-Dioxin column (60m x 0.25mm x 0.25um) (Thermo, USA). The first elution (1,3,6,8-TCDD), column performance (1,2,3,7-TCDD; 1,2,3,8-TCDD) and the last elution (1,2,8,9-TCDD) isomers (from Isotope Cambridge Laboratory) were mixed with label, internal standards of US EPA 1613 method to built-up calibration curves which use to quantitative non-2,3,7,8-TCDD. The average relative response of four specified isomers was applied to another non-2,3,7,8-TCDD. C13-2,3,7,8-TCDD recovery performance was applied for all non-2,3,7,8 TCDD. C131,2,3,4-TCDD was the internal standard for all.

3. Result and Discussion:
3.1. Isomers of 2,3,7,8 PCDD/Fs
The concentration of 2,3,7,8 PCDD/Fs in fly ash samples collected secondary aluminum smelters and waste-to-energy incinerator were shown in table 1. Total concentrations of seventeen toxic PCDD/Fs in fly ash from SAI were ranging between 1893 and 686.3 ng/Kg, corresponding range of 169 and 32.5 ng TEQ/Kg.
The values were lower than those level in Korea and China published by B-W Yu (250-2079 ng TEQ/kg) and T. Chen (268-20400 ng TEQ/kg). The average concentrations of PCDD/Fs in fly ashes were 231.5 ng/Kg, corresponding to 21.04 ng TEQ/kg, which were lower than those result Yun Pan reported (280-190000 ng TEQ/kg).

Table 1: Concentration of seventeen toxic PCDD/Fs in fly ash samples. Amounts in ng/kg

<table>
<thead>
<tr>
<th>Compound</th>
<th>SA1</th>
<th>SA2</th>
<th>WtE1</th>
<th>WtE2</th>
<th>WtE3</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,7,8-TCDD</td>
<td>11.16</td>
<td>1.070</td>
<td>0.5742</td>
<td>0.8448</td>
<td>1.014</td>
</tr>
<tr>
<td>1,2,3,7,8-PeCDD</td>
<td>43.83</td>
<td>4.740</td>
<td>5.144</td>
<td>4.308</td>
<td>5.170</td>
</tr>
<tr>
<td>1,2,3,4,7,8-HxCDD</td>
<td>27.70</td>
<td>3.738</td>
<td>5.810</td>
<td>4.226</td>
<td>5.071</td>
</tr>
<tr>
<td>1,2,3,6,7,8-HxCDD</td>
<td>22.14</td>
<td>5.989</td>
<td>8.693</td>
<td>5.327</td>
<td>6.392</td>
</tr>
<tr>
<td>1,2,3,7,8,9-HxCDD</td>
<td>33.28</td>
<td>1.073</td>
<td>6.363</td>
<td>4.249</td>
<td>5.098</td>
</tr>
<tr>
<td>1,2,3,4,6,7,8-HpCDD</td>
<td>122.4</td>
<td>73.73</td>
<td>10.70</td>
<td>5.666</td>
<td>6.799</td>
</tr>
<tr>
<td>OCDD</td>
<td>164.4</td>
<td>32.33</td>
<td>13.91</td>
<td>11.94</td>
<td>14.33</td>
</tr>
<tr>
<td>2,3,7,8-TCDF</td>
<td>70.72</td>
<td>22.42</td>
<td>5.785</td>
<td>16.80</td>
<td>20.16</td>
</tr>
<tr>
<td>1,2,3,7,8-PeCDF</td>
<td>108.1</td>
<td>30.86</td>
<td>7.200</td>
<td>19.11</td>
<td>22.93</td>
</tr>
<tr>
<td>2,3,4,7,8-PeCDF</td>
<td>145.8</td>
<td>28.60</td>
<td>24.68</td>
<td>17.87</td>
<td>21.44</td>
</tr>
<tr>
<td>1,2,3,4,7,8-HxCDF</td>
<td>153.7</td>
<td>32.52</td>
<td>10.39</td>
<td>13.32</td>
<td>15.99</td>
</tr>
<tr>
<td>1,2,3,6,7,8-HxCDF</td>
<td>148.1</td>
<td>35.71</td>
<td>12.20</td>
<td>14.74</td>
<td>17.69</td>
</tr>
<tr>
<td>1,2,3,7,8,9-HxCDF</td>
<td>58.14</td>
<td>9.590</td>
<td>4.274</td>
<td>1.388</td>
<td>1.665</td>
</tr>
<tr>
<td>2,3,4,6,7,8-HxCDF</td>
<td>113.9</td>
<td>42.59</td>
<td>19.25</td>
<td>15.29</td>
<td>18.35</td>
</tr>
<tr>
<td>1,2,3,4,6,7,8-HpCDF</td>
<td>193.9</td>
<td>87.63</td>
<td>23.26</td>
<td>38.50</td>
<td>46.20</td>
</tr>
<tr>
<td>1,2,3,4,7,8,9-HpCDF</td>
<td>87.17</td>
<td>15.89</td>
<td>3.698</td>
<td>5.464</td>
<td>6.557</td>
</tr>
<tr>
<td>OCDF</td>
<td>388.7</td>
<td>257.9</td>
<td>70.22</td>
<td>31.16</td>
<td>37.39</td>
</tr>
<tr>
<td>Total PCDD/Fs</td>
<td>1893</td>
<td>686.3</td>
<td>232.2</td>
<td>210.2</td>
<td>252.2</td>
</tr>
<tr>
<td>TEQ (ng/kg)</td>
<td>168.9</td>
<td>32.54</td>
<td>21.02</td>
<td>19.13</td>
<td>22.96</td>
</tr>
<tr>
<td>Ratio of PCDDs/PCDFs</td>
<td>3.5</td>
<td>4.6</td>
<td>3.5</td>
<td>4.7</td>
<td>4.7</td>
</tr>
</tbody>
</table>

Figure 1 and Figure 2 showed the homologue patterns of 2,3,7,8 PCDD/Fs in fly ash samples from SA1 smelter and WtE incinerators. PCDFs and highly chlorinated isomers were dominant than other lowly chlorinated congeners, which were seen similar trend in the results given by T. Chen. For PCDD/Fs profile in waste-to-energy incinerators, H6CDF, H7PCDF, P5CDF were higher than all PCDD/Fs and H6CDD was dominant in PCDD group. The PCDF/PCDD ratios were from 3.5 to 4.7, that means total concentration of PCDFs was higher than total PCDD concentrations or de novo reaction was dominant pathway.

3.2. Non-2,3,7,8-TCDD isomers

Table 2 shows the retention times (RT); relative factor (RF), The correlation coefficient (R²) of calibration curves of 1,3,6,8; 1,2,3,7; 1,2,3,8 and 1,2,8,9- TCDD isomers. 1,3,6,8 – TCDD was the first elution congener at 26.51 min and 1,2,8,9-TCDD was the last elution at 35.15 min., and all above TCDD isomers have RT in between 26.51 to 35.15 min. The isomers of 1,2,3,7 and 1,2,3,8-TCCD were the column performance isomers, and they are separated well. All calibration curves have R² > 0.9999. Other isomers use the average of RF from 1,3,6,8; 1,2,3,7; 1,2,3,8 and 1,2,8,9-TCDD to quantitative determination.

Table 2: The retention times (RT); relative factor (RF), The coefficient of determination (R²) of calibration curves of some TCDD isomers

<table>
<thead>
<tr>
<th>isomer</th>
<th>RT (min)</th>
<th>RF</th>
<th>R²</th>
<th>Note</th>
</tr>
</thead>
</table>

...
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<table>
<thead>
<tr>
<th>TCDD-isomer</th>
<th>SA1</th>
<th>SA2</th>
<th>WtE1</th>
<th>WtE2</th>
<th>WtE3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,3,6,8 – TCDD</td>
<td>54.06</td>
<td>45.71</td>
<td>28.34</td>
<td>17.44</td>
<td>27.20</td>
</tr>
<tr>
<td>1,2,3,7 – TCDD</td>
<td>12.24</td>
<td>4.142</td>
<td>7.432</td>
<td>10.93</td>
<td>7.162</td>
</tr>
<tr>
<td>1,2,3,8 – TCDD</td>
<td>12.73</td>
<td>4.334</td>
<td>6.944</td>
<td>9.932</td>
<td>8.252</td>
</tr>
<tr>
<td>1,2,8,9 – TCDD</td>
<td>1.242</td>
<td>2.527</td>
<td>4.167</td>
<td>1.314</td>
<td>0.839</td>
</tr>
<tr>
<td>Total non-2,3,7,8-TCDD</td>
<td>121.9</td>
<td>93.07</td>
<td>81.14</td>
<td>71.38</td>
<td>67.36</td>
</tr>
<tr>
<td>% (2,3,7,8-TCDD/Total TCDD)</td>
<td>9.2</td>
<td>1.1</td>
<td>0.7</td>
<td>1.2</td>
<td>1.7</td>
</tr>
</tbody>
</table>

4. Conclusion:
Five fly-ash samples were collected at SA1 smelters in a recycled aluminum village and WtE incinerator in Northern Vietnam to analyze seventeen 2,3,7,8-PCDD/Fs isomers and total of four other non-2,3,7,8-TCDDs. The total of seventeen toxic 2,3,7,8-substituted PCDD/Fs were ranging between 1893 and 686.3 ng/Kg, corresponding to 169 and 32.5 ng TEQ/Kg; and average 231.5 ng/Kg, corresponding to 21.04 ng TEQ/kg, respectively. High chlorines are dominant, and de novo reaction was the main way to form PCDD/Fs. The percentage value of highest toxic isomer of 2,3,7,8-TCDD was lower than 10% comparison with total TCDD isomers; and 1,3,6,8 TCDD isomer was the highest concentration.

5. Acknowledgments:
This study was funded by component 5: “Improving the quality of dioxin analysis laboratory to international standards”, code TBDIOX.05/22-24, under the key science and technology project of VAST: "Research on the risk of dioxin accumulation and derivatives arising from some socio-economic activities to the food production chain", code: TBDIOX.00/22-24.

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6. References:
5. Mai Thi Ngoc Pham, Anh Quoc Hoang, Xuan Truong Ngiem, Binh Minh Tu, Thi Nhung Dao, Duc Nam Vu, 2019. Residue concentrations and profiles of PCDD/Fs in ash samples from multiple thermal industrial in Viet Nam: Formation, emission levels, and risk assessment. Environmental Science and Pollution Research 17, 17719-17730.
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POPs in Developing Countries
K. Pozo & B. Gevao

MON-PM1-C5 Levels, Humans Health Risk of Triclosan, and Triclocarban in Efluent and Influent Samples from the Selected Wastewater Treatment Plants across Durban, South Africa

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Introduction: Wastewater systems are the primary source of pollution via which pollutants find their way into surface water systems. The pollution associated with wastewater channels is mostly of anthropogenic origin that could result from domestic, industrial, agrochemical, and pharmaceutical sources or a combination of those, which form a major point source of surface or underground water pollution [1,2]. Due to the heterogeneous nature of wastewater, wastewater systems carry various arrays of pollutants through their influent (domestic, industrial, agrochemical, and pharmaceutical) that are complex to handle by many of the wastewater treatment plants (WWTPs). Triclosan (TCS) and triclocarban (TCC) are antimicrobial agents that have been used in personal care and consumer products in the past decades. They are phenolic chlorinated compounds with ether functional groups. These antimicrobials are present in most consumer products they are commonly added to household soaps, detergents, toothpaste, disinfectants, cosmetics, and medical disinfectants for killing/inhibiting microbes, at levels of up to 0.1–0.3% (w/w) and 2% (w/w) of TCS and TCC, respectively [3–4]. Studies have indicated some levels of TCS and TCC have been found in wastewater across the countries. Aimqvist and Hanæus [5] reported levels of TCS in gray water systems from Swedish households in the range of 0.075 µg/L–16.6 µg/L. The fate of TCS and TCC in WWTP influents and effluents have been reported by Bedoux et al. [6], and Tran et al. [6] at levels ranging from 1.3 to 86,200 ng/L and from 3.1 to 5370 ng/L for TCS and TCC, respectively. Therefore, to protect aquatic ecosystems, as well as drinking water supplies, it is important to examine the fate of TCC, and TCS, in the wastewater system as they serve as a major point source of pollution to the surface water, which is the main alternative water source for the larger populations within the Durban metropolis.

Materials and Methods: In this study, influent, effluent, and sludge samples collected in selected wastewater treatment plants across the Durban metropolis were quantified using Gas Chromatography-Mass Spectrometry. Triclosan and Triclocarban standards were purchased from Sigma-Aldrich®, South Africa; DLD Scientific, South Africa, supplied anhydrous sodium sulfate, organic solvents HPLC grade dichloromethane, and acetone (organic solvent). Water physicochemical parameters such as pH, electrical conductivity, total dissolved solids (TDS), and salt were measured on-site using a portable multi-parameter meter (HANNA Instrument Inc., HI 9828 pH/ORP/EC/DO, Woonsocket, RI, USA, made in Romania).

Results and Discussion: The results of this study revealed that TCS and TCC were present in the samples analyzed. It was observed that the concentrations of TCS ranged from 1.906 to 73.462 µg/L, from 1.732 to 6.980 µg/L, and from 0.138 to 2.455 µg/kg in influent, effluent, and sludge samples, respectively. The concentrations of TCC were found to be between 0.320 and 45.261 µg/L, <LOQ–1.103 µg/L, and from 0.107 to 8.827 µg/kg in the influent, effluent, and sludge samples, respectively. The concentrations of TCC were found to be higher in the aqueous samples as compared with those recorded for TCS in the same medium. However, the concentrations of TCS in the sludge samples were significantly higher than the levels recorded for TCS. The trend observed could be due to the higher aqueous solubility of TCS and the more hydrophobicity character of TCC. Another possibility could be that TCS is more frequently applied as an antimicrobial agent in personal care and consumer products. Generally, the mean concentrations of TCS and TCC in influent and sludge samples were both higher than those found in the effluent samples across the treatment plants investigated. In addition, the risk quotient was evaluated in the influent and effluent samples. The RQ values in the influent samples were generally lower than those found in the effluent samples. The RQ value reported for TCS was found to be significantly higher than TCC; therefore, TCS could pose a greater threat to organisms in contact with aquatic environments than TCC. It could be assumed that the treatment processes adopted by the treatment plants were able to remove substantial amounts of TCS and TCC from the raw sewage waste before being discharged to nearby surface rivers, thereby, reducing the ecological risk that could be associated with TCS and TCC in the environment. The RQ values for TCS, TCC, and RI from the Isipingo wastewater influent are 1.75 × 102, 6.036 × 101, and 1.17 × 101, respectively.

Conclusion: The results of this study indicated that substantial amounts of TCS and TCC are been removed during the treatment process which could be a major reason for the decline in the levels recorded in the effluent samples, therefore, reducing the amount of the TCS and TCC that would eventually end up in the surface rivers. It should be noted that the risk quotient (RQ) and hazard index (RI) values are greater than one, which indicates that raw sewage from this wastewater plant finds its way directly into the receiving surface water (Isipingo River). This could lead to a very serious ecological problem or an appreciable risk could exist in the environment.
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MON-PM1-C5  Levels, Humans Health Risk of Triclosan, and Triclocaban in Efluent and Influent Samples from the Selected Wastewater Treatment Plants across Durban, South Africa

Acknowledgments: The authors would like to acknowledge the financial support the research facilities by the Mangosuthu University of Technology for

References:
MON-PM1-D1  Summary of four rounds of interlaboratory assessments on POPs

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² Vrije Universiteit, Amsterdam Institute for Life and Environment, De Boelelaan 1085, 1081 HV, Amsterdam, The Netherlands

1. Introduction:
The objective of the Stockholm Convention (SC) on Persistent Organic Pollutants (POPs) is to protect human health and the environment from POPs with the ultimate goal of their elimination, where feasible (Fiedler et al., 2019; UNEP, 2001). Accurately measuring and analyzing the concentrations of POPs is an important step towards evaluating the effectiveness of the convention and the potential impacts of POPs in humans and the environment. Interlaboratory assessments accompany the United Nations Environment Programme’s (UNEP) capacity building program for laboratories analysing POPs to assess the quality assurance/quality control (QA/QC) practices among laboratories that provide data under the Convention. To determine the ‘true’ concentration of (in this case) POPs in a sample, a chemical laboratory must be able to prove that it is able to identify and quantify chemicals (analytes) of interest at concentrations of interest. As an assessment criterion for satisfactory performance of a laboratory, UNEP has set a margin of ±25% of the consensus value.

To assist laboratories in improving the quality of their analyses, UNEP has organized regional capacity building and training programmes, which started in 2009. As part of this activity, the first round of the global interlaboratory assessment on POPs was organized in 2010-2011 (Abalos et al., 2013; UNEP et al., 2012; van Leeuwen et al., 2013), the second in 2012-2013 (UNEP et al., 2014). The third round was implemented in 2016/2017 (UNEP et al., 2017) and the fourth in 2018/2019 (UNEP et al., 2021). These interlaboratory assessments (IL1-IL4) were coordinated by the Department of Environment & Health of the Vrije Universiteit Amsterdam, the Netherlands (VU E&H) and the Man-Technology-Environment (MTM) Research Center, School of Science and Technology at the University of Örebro, Sweden. A summary of the participation and the performance of the laboratories are discussed in this paper.

2. Materials and Methods:
2.1 POPs and test samples
The number and type of test samples have increased as new POPs were listed in the annexes of the Stockholm Convention; subsequently, new matrices were added. In the last round, the POPs studied included the following POP groups:

- **Organochlorine pesticides (OCPs):** aldrin, dieldrin, endrin, DDT and metabolites, chlordane and metabolites, heptachlor and metabolites, mirex, toxaphene, hexachlorocyclohexanes (HCHs, as –HCH, –HCH, and lindane), chlordcone, endosulfan (as and –endosulfan, endosulfan sulphate);
- **Industrial POPs (indPOPs):** polychlorinated biphenyls (PCB as six indicator PCB), hexachlorobenzene (HCB), pentachlorobenzene (PeCBz), hexachlorobutadiene (HCB);
- **Dioxin-like POPs (dl-POPs):** polychlorinated dibenzo-p-dioxins (PCDD), polychlorinated dibenzofurans (PCDF), dioxin-like PCB; sum parameters as toxic equivalent (TEQ);
- **Brominated flame retardants (BFR):** polybrominated diphenylethers (PBDE), deca-BDE; hexabromobiphenyl (HxBB, as PBB-153), hexabromocyclododecane (HBCD as –HBCD, –HBCD, –HBCD); and
- **Perfluorinated alkyl substances (PFAS):** perfluorooctanesulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorohexanesulfonic acid (PFHxS), PFOS precursors, and a number of PFAS not listed in the Stockholm Convention.

Up to 16 matrices were offered for analysis: nine test solutions to cover all POPs, two air extracts (one in toluene for OCPs, industrial POPs, BFR, and dl-POPs and one in methanol for PFAS), sediment, fish, human milk, human plasma and water (the latter two for PFAS only). The test solutions were amplified in amber glass ampoules with the target compounds in undisclosed concentrations. The air extracts were also amplified, sediment was air-dried, the fish (crab) was sterilized in glass jars, the plasma frozen and the human milk was homogenized, frozen and stored at -20 °C prior to shipment. Water was sent in high-density polyethylene (HDPE) bottles.

2.2 Processing of samples and Results
All participating laboratories were provided with instructions and a template to report results for each of the POP groups electronically (MsExcel®). For each round, all data received were combined into one results database (MsExcel®) according to laboratory (laboratory code), analyte, and test sample. These aggregated data were shared with the participating laboratories for a confirmation of their data and in addition, laboratories were allowed to make small corrections for obvious errors, such
2.3 Assessment of performance

All data received from participating laboratories were assessed according to ISO 13528 (2005, see Thompson et al., 2006). All principles and definitions, such as accuracy and precision, z-scores, reporting of results to the participants, confidentiality, were adhered to. However, the so-called NDA model (normal distribution approximation) was used (Cofino et al., 2005). In addition, the model provides a solution for assigning z-scores to left censored values (LCVs, values below the detection limit). Z-scores \( z = (x_i - X) / \sigma \), in which \( x_i \) is the result of the participant, \( X \) is the assigned value (AV) and \( \sigma \) is the bias, were generated for all results and can be interpreted as follows: \( |z| < 2 \) = satisfactory performance (referred to as ‘S’), \( 2 < |z| < 3 \) = questionable performance (‘Q’), \( |z| > 3 \) = unsatisfactory performance (‘U’), and \( |z| > 6 \) = extreme performance (‘U’; merged with unsatisfactory performance in the assessments). For LCVs the following quality criteria were applied: \( \text{LCV/2} < \) (concentration corresponding to \( |z|=3 \)): LCV consistent with AV (labelled as ‘C’), and \( \text{LCV/2} > \) (concentration corresponding to \( |z|=3 \)): LCV inconsistent with AV (labelled as ‘I’).

3. Results:

In the four rounds of the interlaboratory assessment (IL1-IL4), a total of 82 countries had laboratories registered but laboratories from ten countries did either not deliver any results or did not have any z-score assigned. Figure 1 (left) shows the number of countries by UN region for each IL that had laboratories registered; Figure 1 (right) shows the distribution of the countries that obtained z-scores by round and region. Countries where no results were obtained included Cameroon, Democratic Republic of Congo, Ethiopia, Madagascar, Sudan, Tanzania, Malaysia, Albania, Georgia, and Barbados.

<table>
<thead>
<tr>
<th>Region/Round</th>
<th>IL1 (N=103)</th>
<th>IL2 (N=105)</th>
<th>IL3 (N=176)</th>
<th>IL4 (N=148)</th>
<th>Overall (N=532)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Africa</td>
<td>17</td>
<td>12</td>
<td>19</td>
<td>24</td>
<td>72</td>
</tr>
<tr>
<td>Asia</td>
<td>38</td>
<td>45</td>
<td>68</td>
<td>48</td>
<td>199</td>
</tr>
<tr>
<td>CEE</td>
<td>3</td>
<td>4</td>
<td>23</td>
<td>6</td>
<td>36</td>
</tr>
<tr>
<td>GRULAC</td>
<td>32</td>
<td>14</td>
<td>39</td>
<td>37</td>
<td>122</td>
</tr>
<tr>
<td>WEOG</td>
<td>13</td>
<td>30</td>
<td>27</td>
<td>33</td>
<td>103</td>
</tr>
</tbody>
</table>
Progress in Methods for POPs Analysis

W. Tirler & G. Hunt

MON-PM1-D1  Summary of four rounds of interlaboratory assessments on POPs

Table 2: Overview of number of laboratories by round registered and delivering results

<table>
<thead>
<tr>
<th></th>
<th>IL1</th>
<th>IL2</th>
<th>IL3</th>
<th>IL4</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Registration</td>
<td>103</td>
<td>105</td>
<td>176</td>
<td>148</td>
<td>532</td>
</tr>
<tr>
<td>No result</td>
<td>21</td>
<td>16</td>
<td>43</td>
<td>32</td>
<td>112</td>
</tr>
<tr>
<td>With results</td>
<td>82</td>
<td>89</td>
<td>133</td>
<td>116</td>
<td>420</td>
</tr>
</tbody>
</table>

Table 3: shows the number of participations: 26 laboratories delivered results 4-times and in summary contributed with 104 of the 420 sets of results. Another 31 laboratories participated 3-times; whereas 119 laboratories were one-time participants.

Table 3: Number of participations and sets of delivered results per laboratory

<table>
<thead>
<tr>
<th>No. of rounds with results</th>
<th>No of laboratories</th>
<th>No of sets of results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1x P</td>
<td>119</td>
<td>119</td>
</tr>
<tr>
<td>2x P</td>
<td>52</td>
<td>104</td>
</tr>
<tr>
<td>3x P</td>
<td>31</td>
<td>93</td>
</tr>
<tr>
<td>4x P</td>
<td>26</td>
<td>104</td>
</tr>
<tr>
<td>Total</td>
<td>228</td>
<td>420</td>
</tr>
</tbody>
</table>

In total, 41 575 z-scores were assigned in the four rounds (Table 4). It can be seen that not all the POPs groups were tested from the beginning and that some matrices, such as transformer oil (TO in IL2) or fly ash (Ash as a proxy for the air extract in IL1) were included only once. The numbers of z-scores were largest in IL3, due to the largest number of participating laboratories. The test solutions of the analytical standards (Test_soln) always had the highest number of results (>15 000). Among the POP groups, most z-scores were attributed to the dl-POPs (19 500), followed by the OCPs (9 526). Strong increases between rounds were observed for BFR and PFAS.

Table 4: Summary of z-scores by round, region, type of test sample, and POP-group

<table>
<thead>
<tr>
<th>Region</th>
<th>IL1 (N=6 464)</th>
<th>IL2 (N=10 491)</th>
<th>IL3 (N=13 255)</th>
<th>IL4 (N=11 365)</th>
<th>Overall (N=41 575)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Africa</td>
<td>394</td>
<td>191</td>
<td>1 054</td>
<td>656</td>
<td>2 295</td>
</tr>
<tr>
<td>Asia</td>
<td>3 522</td>
<td>5 149</td>
<td>5 884</td>
<td>4 589</td>
<td>19 144</td>
</tr>
<tr>
<td>CEE</td>
<td>154</td>
<td>512</td>
<td>1 401</td>
<td>363</td>
<td>2 430</td>
</tr>
<tr>
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<td>948</td>
<td>695</td>
<td>1 338</td>
<td>1 587</td>
<td>4 568</td>
</tr>
<tr>
<td>WEOG</td>
<td>1 446</td>
<td>3 944</td>
<td>3 578</td>
<td>4 170</td>
<td>13 138</td>
</tr>
<tr>
<td>Type</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Test_soln</td>
<td>2 434</td>
<td>3 542</td>
<td>4 834</td>
<td>4 202</td>
<td>15 012</td>
</tr>
<tr>
<td>Abiotic (air, sediment, water, ash, TO)</td>
<td>2 175</td>
<td>4 113</td>
<td>4 847</td>
<td>4 822</td>
<td>15 957</td>
</tr>
<tr>
<td>Biota (human milk, fish, HP)</td>
<td>1 855</td>
<td>2 836</td>
<td>3 574</td>
<td>2 341</td>
<td>10 606</td>
</tr>
<tr>
<td>POP_group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OCPs</td>
<td>1 599</td>
<td>2 160</td>
<td>3 377</td>
<td>2 390</td>
<td>9 526</td>
</tr>
<tr>
<td>indPOP</td>
<td>966</td>
<td>1 716</td>
<td>2 202</td>
<td>1 497</td>
<td>6 381</td>
</tr>
<tr>
<td>dl_POPs</td>
<td>3 899</td>
<td>5 121</td>
<td>5 897</td>
<td>4 613</td>
<td>19 530</td>
</tr>
<tr>
<td>BFR</td>
<td>0</td>
<td>1 074</td>
<td>1 149</td>
<td>996</td>
<td>3 219</td>
</tr>
<tr>
<td>PFAS</td>
<td>0</td>
<td>420</td>
<td>630</td>
<td>1 869</td>
<td>2 919</td>
</tr>
</tbody>
</table>
The quality of the z-scores is summarized in tabular form and visualized in Figure 2. It can be seen that most z-scores were assigned to Asian laboratories and that 64% of these z-scores were satisfactory and only 23% unsatisfactory. An even better ration but with less results was obtained for the WEOG laboratories that had 69% satisfactory and only 16% unsatisfactory. For the African laboratories the assessment is very disappointing since 59% of the z-scores were unsatisfactory and just 21% satisfactory. Further, they had the largest amount of LCV values (as “I”), i.e., that their LODs were inconsistent with the assigned values. This finding indicates that the African laboratories have problems with the sensitivity and that the actual concentrations in the test samples were too low to be quantified by them. These problems were found for all types of test samples; whereby it was noted that overall, the best results were obtained for the Test solutions followed by Abiotic. The biota samples caused more problems (Figure 3).

Finally, among the POPs, the dioxin-like POPs (PCDD, PCDF, dl-PCB, TEQ_DF, TEQ_PCB, and TEQ_total but also toxaphene, HBCD, sum_PBDE, PFOS, PFOA, and PFHxS posed less problems to the often specialized laboratories than some of the legacy OCPs, such as especially ∼-HCH, -HCH, and lindane, endosulfan or the drins (as single compounds and as sum). The results for PBDE_209, which was included only in IL4 were also disappointing (Figure 4).

<table>
<thead>
<tr>
<th></th>
<th>S</th>
<th>Q</th>
<th>U</th>
<th>C</th>
<th>I</th>
</tr>
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<tbody>
<tr>
<td>Africa</td>
<td>21%</td>
<td>9%</td>
<td>59%</td>
<td>1%</td>
<td>11%</td>
</tr>
<tr>
<td>Asia</td>
<td>64%</td>
<td>9%</td>
<td>23%</td>
<td>1%</td>
<td>3%</td>
</tr>
<tr>
<td>CEE</td>
<td>51%</td>
<td>9%</td>
<td>32%</td>
<td>1%</td>
<td>6%</td>
</tr>
<tr>
<td>GRULAC</td>
<td>45%</td>
<td>9%</td>
<td>37%</td>
<td>2%</td>
<td>7%</td>
</tr>
<tr>
<td>WEOG</td>
<td>69%</td>
<td>11%</td>
<td>16%</td>
<td>2%</td>
<td>2%</td>
</tr>
</tbody>
</table>

Figure 2: Stacked bars showing number of z-scores by region colored by quality of the z-score (N=41,575)

Figure 3: Stacked bars showing number of z-scores by type and colored by z-score (N=41,575)
4. Discussion:
The organization and execution of international interlaboratory assessments has provided insights into the existence and performance of POPs laboratories. First, all laboratories that participated must be applauded for its willingness to undergo such comparative projects. The outcomes, however, showed that a number of laboratories have overestimated their capacities and capabilities since they were not able to deliver results and obtain z-scores (see Table 2, number of laboratories that did not deliver results). From the z-scores, we could determine regional gaps such as that in Africa and GRULAC, there was only one laboratory in each region, capable to analyze PFAS and for BFR, there were only one or three labs, resp. On the other hand, there was a high number of qualified laboratories for all POPs in China. The interlaboratory assessments have shown that there was only one laboratory that analyzed all POPs and all matrices; all other were specialized to either POPs or matrices. Specialization depended on instrumentation, e.g., the majority of the chlorinated and brominated POPs were analyzed by gas chromatographs coupled to either electron capture (ECD) detectors or mass selective (MS) detectors. It must be stated that GC/ECD combinations in most cases gave poorer results than GC/MS. For dl-POPs, the sector-field high resolution instruments gave the best results and were commonly used. PFAS but also isomer-specific determination of HBCD require high-performance liquid chromatographs with MS or MS/MS detection; thus, a different instrumentation; these laboratories showed overall good performance.

5. Conclusions:
The four rounds of interlaboratory assessments have generated an abundance of information, which can be further assessed. It is recommended that one-time participating laboratories evaluate their performance with a view on their ambitions and identify strengths and weaknesses in comparison with other laboratories in the same country or the region. For laboratories with multiple participation, the results should be evaluated with a view on the performance, such as if the laboratory has become more proficient with time; not only in terms of the ratio S/U results but also if more POPs or more matrices could be analyzed in the more recent rounds. It is highly recommended that such proficiency tests are organized annually or at least biennially as part of a QA/QC programme.

6. Acknowledgments:
The interlaboratory assessments were founded by UNEP with funds provided by the Global Environment Facility (GEF, Washington, DC, USA), the European Commission, the Quick Start Programme of SAICM, and by the laboratories from OECD countries (self-funded).

7. References:
MON-PM1-D1: Summary of four rounds of interlaboratory assessments on POPs


MONT-PM1-D2  Assessment of laboratory indoor air contamination in ultra-trace analysis of ubiquitous organic contaminants: Towards a better QA/QC

Ingrid Guiffard¹, Gaëlle Raffy², Julien Sinquin³, Charles Pollono¹, Philippe Marchand¹, Emilie Surget⁴, Javier Castro-Jiménez⁵, Fabien Mercier², Emmanuelle Bichon¹

¹Oniris, INRAE, LABERCA, Nantes
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³Ifremer, CCEM, Nantes

Introduction: Laboratories analyzing legacy or emerging contaminants are confronted with the contamination of their indoor environment by these same contaminants. In parallel, current analytical instruments reach such sensitivities that ubiquitous compounds are systematically detected. The indoor environment of laboratories (gaseous and particulate phases of the air, and settled dust) can be a significant source of contamination in the final extract, impacting the limits of quantification and the final quality of the analysis. Moreover, laboratories need to conduct their analyses under known, stable and controlled conditions, which can be tricky with some contaminants. Today, this issue is not well addressed in the literature, although the analytical and environmental sciences communities are increasingly confronted with it.

Our project gathered three laboratories to conduct a joint study on the nature and levels of contamination of their workplaces to better understand ultra-trace level measurements, based on their complementary skills and the diversity of their sites. The families of compounds targeted by this project are synthetic musks, brominated flame retardants (BFRs) such as BDEs including decaBDE, DBDPE and HBCDDs, organophosphorus flame retardants and plasticizers (OPFRs), phthalates esters (PAEs), per- and polyfluoroalkyl substances (PFASs), organochlorine pesticides (OCPs) and polycyclic aromatic hydrocarbons (PAHs).

Materials and Methods: Two sampling campaigns were conducted over a six-month period (September-October 2022 and March 2023). For each campaign, low-volume air samplers gathered 20 m³ (one week), which passed first through quartz fiber filters (QFFs) and then polyurethane foams (PUF), in selected areas of each laboratory subject to different uses and potential sources of organic contaminants. In addition, during the second campaign, dust samples were collected using a damp wipe on a normalized surface of 0.1 m². Air samples (filters and foams) were extracted by pressurized liquid extraction with dichloromethane for the analysis of musks, BFR, OPFR, PAEs, OCPs and PAHs by GC/MS/MS, or with methanol for the analysis of HBCDDs and PFAS by LC/MS/MS, whereas dust samples were extracted from wipes with microwaves assisted extraction with a DCM/Acetone mixture (67:33, v/v) before analysis using the same way as for the air samples. In the first campaign, gas and particles were analyzed together, while in the second campaign the gaseous phase of the air (PUF foam), and the particulate phase of the air (QFFs) were analyzed separately.

Results: on PAHs are presented here, as proof of concept, along with data on selected emerging contaminants. PAH concentrations in ambient air ranged from 1 to 5 ng/m³ depending on the laboratories and zones concerned (with the lowest levels observed in rooms equipped with overpressure air systems), with a contribution mainly from phenanthrene (the most volatile compound of our PAH list) compared to the other PAHs investigated (the more volatile PAHs such as naphthalene, acenaphthene and acenaphthyene were not monitored in this study).

Discussion and Conclusion: These values are homogeneous across the 3 laboratories and tend to be lower than the levels already described in indoor environment in the literature (Wei et al., 2019; Wei et al., 2021). The heaviest compounds are weakly represented in indoor air, but the particulate phase on its own and the settled dust remain to be investigated. Thus, the PAH partitioning in the indoor environment can help us to understand the contamination sources, by analyzing separately the various indoor air phases (i.e. gaseous, particulate and settled dust). This way, specific measures could then be recommended to reduce the pressure of contamination on the analytical batches, based on cleaning procedure, dedicated rooms and material, or specific equipment.

References:
**MON-PM1-D3  Incorporation of EPA1613 HRMS Instrument Performance Quality Controls into Tandem Quadrupole MS/MS Methods**

Gareth Rhys Jones*

1Waters Corporation, Wilmslow, SK9 4AX, United Kingdom

**Introduction:**
Tandem quadrupole mass spectrometers (MS/MS) are being used more frequently for the analysis of dioxins, furans and dioxin-like PCBs, with changes to European legislation to accommodate the use of these as an alternative to the more traditional High Resolution Mass Spectrometry (HRMS). There has also been recent interest in broadening the scope of the US EPA 1613 regulations to allow the use of tandem quadrupoles.

The EPA 1613 method outlines several mass spectrometry quality control measures to ensure to integrity of the analysis, developed around the use of high-resolution magnetic sector mass spectrometer utilizing an electron ionization (EI) source. In this study, several techniques are presented to provide analogous quality control measures when using tandem quadrupole mass spectrometry to perform the analysis, using both electron ionization and atmospheric pressure chemical ionization (APCI) sources.

**Materials and Methods:**
Data were acquired using a Waters APGC™ Xevo™ TQ-XS™ tandem mass spectrometer, coupled to an Agilent 8890 GC Oven. Separations were performed with a Restek Rtx-Dioxin2 GC column. Calibration standards were obtained from Wellington Laboratories. MS method development was performed on MassLynx™ 4.2.

**Results:**
The modified MS/MS analytical method records the mass spectral peak shape for both quadrupole mass analyzers, for reference material ions that bracket the mass range of the experiment. This provides a record of mass accuracy and resolution, which are stored as part of each sample acquisition. This is performed during the period preceding the solvent front of the GC separation and so does not incur any additional time burden during batch analysis.

An additional experiment function is incorporated into the modified method, which is used to monitor a reference material mass peak throughout the run. This provides a record of the consistency of the source's ionization efficiency during the GC separation and monitor for any ionization suppression events, which are primarily due to the elution of high concentration matrix components. This satisfies a key quality control requirement of the EPA1613 method and can highlight when further sample cleanup may be required.

Isotopic ratios for all PCDD/Fs and PCBs in a MS/MS experiment will differ from those that are measured by HRMS. This is because the neutral fragment lost in the collision stage between the two quadrupole analyzers includes a chlorine atom, which is always assumed to be a $^{35}$Cl atom. The technique for calculating the theoretical isotope ratios in an MS/MS analysis is explained in this study, and the measurement precision and accuracy expectations are discussed.

The revised method also includes additional MS/MS transitions to target the tertiary isotopes for all components. This quality control enhancement is not required for equivalence to the EPA1613 method, but it does provide further MS diagnostics and allows some flexibility if an interfering chromatographic peak is encountered.

**Discussion and Conclusion:**
Tandem quadrupole mass spectrometry analytical methods can be adapted to provide equivalent quality control measures to those used for magnetic sector HRMS instruments for the targeted analysis of dioxins and furans following the US EPA1613 method. The flexibility of the MS/MS instrument can be used to include additional checks of instrument performance.

**References:**
1. Commission Regulation (EU) 589/2014, laying down methods of sampling and analysis for the control of levels of dioxins, dioxin-like PCBs and non-dioxin-like PCBs in certain foodstuffs.
1. Introduction:
While in the past foods and animal feeds were mainly analyzed only for polychlorinated dioxins, dibenzofurans, and polychlorinated biphenyls, the analytical spectrum continues to expand. This can also be seen in the extension of the scope of the European Union Reference Laboratory for Halogenated Persistent Organic Pollutants in Feed and Food (EURL POPs). In addition to the known groups of dioxins and PCBs, the scope was also extended in 2018 to include polychlorinated naphthalenes and polybrominated flame retardants such as polybrominated diphenyl ethers (PBDEs) or the so-called “emerging brominated flame retardents” (emerging BFRs). The demand for analyses of brominated and mixed halogenated dioxins is also increasing. The need for rapid and high-throughput methods to identify and confirm non-compliant samples in the feed and food chain is becoming increasingly important. Sample processing is still performed manually in many laboratories. Purification from fat extraction to final extract can take several days. In dioxin and PCB analysis, automated multi-column purification on a sulfuric acid silica gel column, an aluminum oxide column and finally on a carbon column has been established. Depending on the column configuration, different fraction results can be achieved. Automated purification usually takes only 60 to 90 minutes. In addition to dioxins and PCBs, the other chlorinated and brominated pollutants that are stable to sulfuric acid can also be determined in the fractions.

2. Materials and Methods:

Samples:
The samples were taken by local authorities as part of food surveillance and national control plans.

Reagents:
Native and \(^{13}\)C-labelled PCDD/F, PBDD/F, PCB, PBDE and PCN standards were purchased from Promochem, Germany or Campro Scientific, Germany.
Solvents used were of quality grade "Nanograde" and purchased from Promochem, Germany.

Apparatus:
DexTech Plus system from LCTech Germany with sulfuric acid/silica gel column, alumina column and active carbon column.

GC-HRMS: Agilent HP 6890/Micromass AutoSpec Ultima HRMS and Thermo Scientific Trace GC/ DFS.

Extraction procedures:
Different amounts of dry food or feed are mixed with sodium sulfate, placed into a glass fiber cartridge and extracted in a Soxhlet extractor with toluene/acetone 70/30 for 4-5 hours. Internal standards are added to dry samples prior to extraction.
Foods of animal origin are mixed with sodium sulfate and glass granulate, placed into a glass column and extracted with a mixture of cyclohexane/dichloromethane 1:1. The internal standards are added to an aliquot of the fat, normally 2 g, which can be extended to 4 g.

Automatic clean-up with the DexTech Plus system from LCTech Germany.
The extracted samples are redissolved in 1 ml acetone, the containers filled with cyclohexane to 10 ml and loaded directly into the auto sampler of the system. By using toluene in the extraction of feed and dry food and by adding the internal standard in toluene to the fat, there is up to 0.5 ml of toluene in the solution. The vials are washed three times with 1 ml cyclohexane. The processing takes place in the DexTech Plus with the standard configuration for an aluminum oxide column. It starts with a sulfuric acid column. For the workflow described here, it makes no difference whether the standard column or universal column is used. The substances are washed with cyclohexane from the acidic silica gel column onto the alumina column. From there, the substances are flushed with cyclohexane/dichloromethane onto a small column of activated carbon. Substances that are not retained by the activated carbon are collected as fraction 1. The retained substances are eluted with toluene in fraction 2 from the activated carbon column.

GC/MS Analysis:
a) Agilent 6890 GC/Micromass Autospec Ultima HRMS: Carrier gas: helium, pressure: 2 bar; MS-Resolution: R=10,000; PCDD/F/non ortho PCB and PCN: Column: DB-5MS (Agilent) 60 m, 0.10 mm film thickness, 0.25 mm ID; Injector: 275 °C, 2 µl splitless; Temperature program (PCDD/F/PCB): 100 °C (2 min) - 165 °C (20 °C/min) - 290 °C 4 °C/min); Temperature program (PCN): 100 °C (2.5 min) - 170 °C (35 °C/min) - 290 °C 4 °C/min.)
MON-PM1-D4  Multimethod for the determination of halogenated organic pollutants in food and feed

b) Trace GC/DFS Thermo Scientific, Bremen: Carrier gas: helium, pressure: 2 bar; MS-Resolution: R=10,000
Mono- and di-ortho PCB: Column: HT-8 (SGE) 50 m, 0.25 mm film thickness, 0.25 mm ID; Injector: 280 °C, 1 µl splitless; Temperature program: 105 °C (3 min) - 195 °C (30 °C/min) - 315 °C (4 °C/min)
PBDE/ PBDD/F and PXDD/F/emerging BFR: Column: HP-5MS (Agilent) 25 m, 0.11 mm film thickness, 0.20 mm ID; Injector: 280 °C, PBDE: 2 µl splitless; Temperature program: 100 °C (2.5 min) - 160 °C (20 °C/min) - 320 °C (9 °C/min)
PBDD/F and PXDD/F: 1 µl splitless; Temperature program: 100 °C (2.5 min) - 140 °C (40 °C/min) - 320 °C (10 °C/min)
Emerging BFR: 1 µl splitless; Temperature program: 80 °C (2.5 min) - 280 °C (30 °C/min) - 300 °C (10 °C/min)

3. Results:
With the method described, two fractions are obtained. The first fraction has a volume of 24 ml cyclohexane/dichloromethane 1:1. The second fraction consists of 10 ml toluene. At present, the analytes described in table 1 can be determined.

<table>
<thead>
<tr>
<th>Fr. 1</th>
<th>Polychlorinated biphenyls</th>
<th>mono-ortho PCB</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>di-ortho PCB</td>
</tr>
<tr>
<td></td>
<td>Polybrominated diphenylethers</td>
<td>PBDE 28, 47, 49, 99, 100, 153, 154, 183, 209</td>
</tr>
<tr>
<td></td>
<td>Hexabromobenzene</td>
<td>HBB</td>
</tr>
<tr>
<td></td>
<td>1,2-bis(2,4,6-tribromophenoxy)ethane</td>
<td>BTBPE</td>
</tr>
<tr>
<td></td>
<td>Pentabromomethylbenzene</td>
<td>PBE</td>
</tr>
<tr>
<td></td>
<td>Pentabromotoluene</td>
<td>PBT</td>
</tr>
<tr>
<td></td>
<td>1,2,4,5-tetrabromo-3-6-dimethylbenzene</td>
<td>TBX</td>
</tr>
<tr>
<td></td>
<td>Pentabromobenzene</td>
<td>PBBz</td>
</tr>
<tr>
<td>fr. 2</td>
<td>Polychlorinated dibenzo-p-dioxins and dibenzofurans</td>
<td>PCDD/F</td>
</tr>
<tr>
<td></td>
<td>polychlorinated dibenzo-p-dioxins and dibenzofurans</td>
<td>PBDD/F</td>
</tr>
<tr>
<td></td>
<td>Mixed halogenated dibenzo-p-dioxins and dibenzofurans</td>
<td>PXDD/F</td>
</tr>
<tr>
<td></td>
<td>Polychlorinated naphthalenes</td>
<td>PCN -27, 28/36, 42, 46, 48, 49, 50, 52/60, 53, 64/68, 65, 66/67, 69, 70, 71/72, 73, 74, 75</td>
</tr>
<tr>
<td></td>
<td>Decabromodiphenylethane</td>
<td>DBDPE</td>
</tr>
</tbody>
</table>

In routine analysis, the mono- and di-ortho PCBs and the PBDEs are determined as standard in the first fraction. In addition, some of the emerging BFRs are found in this fraction too. The problem with emerging BFRs is that a 13C-labelled ISTD is not available for all substances of interest. Quantification is therefore always associated with greater uncertainty. The final volume of this fraction is usually 100 µl, which is completely sufficient for the concentration range of mono- and di-ortho PCBs. Since especially the low-chlorinated PCBs are highly volatile, the addition of a keeper is advantageous. In the CVUA-MEL, dodecane is usually used for this purpose. However, the keeper must not make up more than 10% of the final solution in order to avoid a major chromatographic effect. For PBDE analysis as well as for the analysis of emerging BFRs, a smaller final volume is necessary, and it is advisable to dispense with a keeper. To measure the substances in fractions, three injections are required with high-resolution sector field mass spectrometry.

Fraction 2 contains the chlorinated, brominated and mixed halogenated dioxins, the non-ortho PCBs and the PCNs. The chlorinated dioxins, the non ortho PCBs and the PCNs are analyzed routinely. For this purpose, the fraction is injected twice. The chromatography is carried out both times on the same column, only using a different temperature program. The brominated dioxins and the mixed halogenated dioxins are analyzed on a different separation column. To increase sensitivity, the remaining extract is further concentrated and only one further injection is possible.
Overview chromatograms of four of the analyte groups (PBDE; PBDD/F; PCN and emerging BFR) are shown in Figure 1.

Figure 1: Overview TIC-chromatograms of PBDE, PCN, PBDD/F and emerging BFR

4. Discussion:
The suitability of this method was tested in a cod liver oil (2021) and fish oil samples A and B (2022/2023) proficiency tests (PT) organized by EURL POPs. The results of the CVUA-MEL as well as the assigned values of the PT and the achieved z-scores are listed for the PBDE and emerging BFRs in table 2.

Table 2: CVUA-MEL results, assigned values and z-scores of the PT of the EURL POPs: Cod liver oil and fish oil samples A and B

<table>
<thead>
<tr>
<th>Polybrominated diphenyl ethers (PBDEs)</th>
<th>EURL PT Cod Liver Oil 2021</th>
<th>EURL PT Fish Oil A 2022/23</th>
<th>EURL PT Fish Oil B 2022/23</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>result µg/kg fat</td>
<td>Assigned values µg/kg fat</td>
<td>Z-scores</td>
</tr>
<tr>
<td>2,2′,4-tribromodiphenyl ether (BDE-28)</td>
<td>0.064</td>
<td>0.072</td>
<td>-0.6</td>
</tr>
<tr>
<td>2,2′,4,4′-tetrabromodiphenyl ether (BDE-47)</td>
<td>1.17</td>
<td>1.28</td>
<td>-0.4</td>
</tr>
<tr>
<td>2,2′,4,5-pentabromodiphenyl ether (BDE-99)</td>
<td>0.175</td>
<td>0.196</td>
<td>-0.5</td>
</tr>
<tr>
<td>2,2′,4,6-pentabromodiphenyl ether (BDE-100)</td>
<td>0.323</td>
<td>0.338</td>
<td>-0.2</td>
</tr>
<tr>
<td>2,2′,4,5,5′-hexabromodiphenyl ether (BDE-153)</td>
<td>0.046</td>
<td>0.057</td>
<td>-1</td>
</tr>
<tr>
<td>2,2′,4,5,6′-hexabromodiphenyl ether (BDE-154)</td>
<td>0.221</td>
<td>0.247</td>
<td>-0.5</td>
</tr>
<tr>
<td>2,2′,3,4,5,5′-heptabromodiphenyl ether (BDE-183)</td>
<td>0.0071</td>
<td>0.01</td>
<td>0.0069</td>
</tr>
<tr>
<td>2,2′,3,4,4′,5,5′,6,6′-decaabromodiphenyl ether (BDE-209)</td>
<td>0.139</td>
<td>0.209</td>
<td>0.27</td>
</tr>
<tr>
<td>Sum of 8 PBDEs without BDE-209 (upper bound)</td>
<td>2.38</td>
<td>2.65</td>
<td>-0.5</td>
</tr>
<tr>
<td>Sum of 8 PBDEs without BDE-209 (lower bound)</td>
<td>2.38</td>
<td>2.64</td>
<td>-0.5</td>
</tr>
<tr>
<td>Sum of 9 PBDEs including BDE-209 (upper bound)</td>
<td>2.52</td>
<td>2.85</td>
<td>-0.6</td>
</tr>
<tr>
<td>Sum of 9 PBDEs including BDE-209 (lower bound)</td>
<td>2.52</td>
<td>2.69</td>
<td>-0.3</td>
</tr>
<tr>
<td>PBBz (1,2,3,4,5-Pentabromobenzene)</td>
<td>0.307</td>
<td>0.317</td>
<td>0.216</td>
</tr>
<tr>
<td>HBB (1,2,3,4,5,6-Hexabromobenzene)</td>
<td>0.304</td>
<td>0.336</td>
<td>0.187</td>
</tr>
<tr>
<td>PB (1,2,3,4,5-Pentabromo-4-methylbenzene)</td>
<td>0.348</td>
<td>0.298</td>
<td>0.255</td>
</tr>
<tr>
<td>PBEB (1,2,3,4,5-Pentabromo-6-ethylbenzene)</td>
<td>0.317</td>
<td>0.305</td>
<td>0.242</td>
</tr>
<tr>
<td>BTBPE (1,1′-(1,2-Ethanediylbis[oxo,bis(2,5-dibromo)]benzene)</td>
<td>1.18</td>
<td>0.745</td>
<td>1.65</td>
</tr>
<tr>
<td>TBE (1,2,4,5-Tetraabromobenzene)</td>
<td>0.285</td>
<td>0.3</td>
<td>0.236</td>
</tr>
<tr>
<td>DOBPE (1,1′-(1,2-Ethanediylbis[2,4,5,6-tetraabromobenzene])</td>
<td>2.26</td>
<td>0.874</td>
<td>1.17</td>
</tr>
</tbody>
</table>
The results for the PCN were only evaluable for the matrix cod liver oil at the time. At the end of the first round of the PT of fish oil there were not enough results available for an assessment of the fish oil samples. However, the results for the CVUA-MEL for the cod liver oil were always close to the mean value. The z-scores ranged from -0.2 to 0.6 for those parameters for which an assigned value could be calculated. For the comparability of PCN results it is important to agree on a list of parameters. As there is also some overlap of PCN congeners, the priority list for the PCNs of the EURL POPs should be followed. The results, also in comparison with the other laboratories, confirm the internal validation of the method. However, the significance for brominated and mixed halogenated dioxins is limited, as proficiency tests are hardly offered worldwide, and the number of laboratories which perform testing of these analytes is also quite small. Recent publications in the field of recycled plastics show that the substances described here are increasingly being placed on the market in recycled products and that the probability of these recycled materials being used in food contact materials is growing.

5. Conclusions:
The method described here provides a powerful tool to establish a multimethod for the determination of various halogenated classes of organic pollutants in feed and food. Certainly, some problems still require resolution. For example, some interesting substances, such as hexabromocyclododecanes (HBCDD) remain on the sulfuric acid column and can only be eluted from there with dichloromethane. Furthermore, additional 13C-labelled standards are needed. Nevertheless, the flexibility of the method described here shows that these problems can be solved, and the method can also be extended to the analysis of other halogenated contaminants.

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F. Arcadio¹², R. Pitruzzella¹, G. D’Agostino⁰, R. Rovida¹, C. Perri¹, A. Chiodi², S. Moretti³, G. Porto⁴, G. Brambilla⁴, L. Zeni¹, N. Cennamo¹²

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Introduction: Perfluorooalkyl Substances (PFASs) are a broad category of chemical substances whose negative effects on both the environment and human health have been extensively documented. Recently, many countries have already issued restrictive laws, imposing very low limits, especially for PFOA in drinking water and foods. Considering this new legislation, a novel ultra-sensitive PFAs sensor was designed, built and experimentally tested. The proposed sensor is based on Plastic Optical Fibres (POFs) platforms in which a specific synthetic receptor was used. Specifically, a Molecularly Imprinted Polymer (MIP) for PFOA is coupled with the POFs-based transductor. In this sensor system, the shift in resonance wavelength is not attributed to a variation of the MIP refractive index over the plasmonic surface but to the MIP refractive index variation into the core of the POF of a chemical chip [1]. This is possible thanks to the properties of multimodal fibres and the series of an SPR-POF platform with a MIP-based chemical chip [1]. Firstly, the sensor was tested with standard solutions of increasing PFOA concentration to assess the sensor response. Then, real liquid samples were processed. The obtained results were compared with results from traditional analytical techniques, such as Liquid Chromatography coupled with Mass Spectrometry (LC-MS and LC-MS-MS), run on the same samples.

Materials and Methods: Perfluorooctanoate ammonium salt (PFO-NH₄), (Vinylbenzyl) trimethylammonium Chloride (VBT), 1H,1H,2H,2H-perfluorodecyl acrylate (PFDA), 2,2-azobisisobutyronitrile (AIBN) and Ethylene Glycol Dimethylacrylate (EGDMA) were used for MIP synthesis. Ultrapure water (Milli-Q®, Merck KGaA, Darmstadt, Germany) was used as solvent. Standard solutions were prepared by dilution from a 40 ppm PFOA stock solution in ultrapure water.

Experimental set up: all measurement were performed with Spectra760 pro instrument (Moresense srl, Milan, Italy).

Results: The sensor response, derived from dose-response curves made with standard solutions, ranges from 1 ng/l to 20000 ng/L. Figure 1 shows normalized SPR spectra from one dose-response curve experiment.

Discussion and Conclusion: The sensor’s working range is wider than the one obtained in a previous study [1] and similar to other approaches that do not rely on MIP-SPR-POF configuration. Since the good experimental results, the presented sensor is very promising for PFOA sensing in a real scenario and a cheaper, reproducible, less time-consuming alternative to traditional techniques. Tests made on real samples yielded the same PFOA concentration as LC-MS analysis. Moreover, due to the low-cost and small-size of the instruments, in situ analysis could be performed. Future studies will focus on other complex matrixes, such as food.

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Early Life Exposure to Endocrine Disruptors: Key Learnings from Metabolome Mapping of Rat Models

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Introduction: Exposure to endocrine-disrupting chemicals (EDCs) in humans during pregnancy and early stages of life can impair normal brain development and reproductive function patterns, leading to severe pathologies later in life. Studies have shown developmental neurotoxicity effects of bisphenols (BPA mostly) and phthalates [1],[2]. However, for many chemicals, such as pyrethroids, per- and polyfluoroalkyl substances (PFAS), organophosphate flame retardants, and plasticizers, hardly any information is available on potential neurodevelopmental effects. Metabolomics has shown to be a powerful tool in environmental toxicology, and key functions related to steroid and thyroid hormones, neurotransmitters, lipids and brain development makes them suitable for discovery analysis and as potential candidate biomarkers for neuroendocrine toxicity in animals and humans. We, therefore, applied targeted and untargeted metabolomics to map the metabolic pathways affected by six EDCs, namely bisphenol F (BPF), permethrin (PMT), butyl benzyl phthalate (BBzP), triphenyl phosphate (TPHP), perfluorooctane sulfonic acid (PFOS), and 1,2-cyclohexane dicarboxylic acid diisononyl ester (DINCH) in rats exposed during pregnancy and lactation. This study was specifically designed to detect and extend all possible metabolic endpoints relevant for assessing the neuro-endocrine axis caused by EDCs mediated at the early stages of life and later life effects such as behaviour changes and cognition.

Materials and Methods: Female and male parent (F0) rats were exposed to BPF, PMT, BBzP, TPHP, PFOS, and DINCH (single exposure) through diet: the dams were exposed during pre-mating, mating, pregnancy, and lactation; the pups were exposed until postnatal day 6 (PND 6) via maternal milk. Behaviour including learning and memory of grownup pups was recorded. Samples of plasma from adult rats and hippocampus from F1 pups (PND6) were collected. Liquid chromatography combined with high-resolution mass spectrometry (LC-ESI QTOF) was used for targeted lipidomics and metabolomics analysis, while liquid chromatography combined with tandem mass spectrometry (LC-MSMS) was used for targeted steroidomics, thyroid hormones and neurotransmitters analysis. Untargeted metabolomics and lipidomics were processed with various tools (e.g. MS-DIAL or Metaboscape) and data was normalized and scaled (Noreva or Metaboanlayst) before statistical analysis. Relationships between exposure and the changes in specific metabolite pathways were performed through GraphPad prism or R.

Results: Steroidomics results showed that steroids were affected both in PND6 hippocampus and adult plasma when comparing the control group and the exposure groups, and effects were gender and chemical-specific. For example, corticosterone was downregulated by BPF, PMT, BBzP and TPHP and upregulated by PFOS in F1 male hippocampus, while in F1 females it was downregulated by BPF, PMT and BBzP. T4 in females was downregulated by PMT and by PFOS in males. Estrone (E1) was downregulated in males exposed to PMT and upregulated in males exposed to BPF; 17 ∼-E2 was downregulated after PMT (both females and males), and after PFOS and TPHP exposure (females), and upregulated by BPF in males only. Strong effects on pregnenolone sulfate, which is linked to cognition, were found in the hippocampus. Lipids metabolism were affected by all 6 EDCs, and sex-specific effects were found. In general, more lipids were regulated in males than in females by the EDCs.

Discussion and Conclusion: EDCs are known to influence the steroid & thyroid hormone pathways, and their mode of action can interfere with/or mimic the functions and signaling of these hormones in the target organs. However, these hormonal pathways are influencing or are under the influence of a wider network of signaling molecules, all contributing to a proper brain development and metabolism. It has been shown that steroids can affect brain’s neurotransmitter systems and that glucocorticoids in the liver regulate sugars and lipid metabolism. Lipid metabolism in the brain is tightly regulated to maintain the neuronal structure and function and may signal nutrient status to modulate metabolism in peripheral tissues such as the liver. By mapping the metabolome of a one-generation exposure study to different chemicals, we aim to cover all the key metabolic endpoints needed for assessing neurotoxicity of EDCs mediated at early developmental stages. These findings will be primarily useful to define mode of action of EDCs on developing neuro-endocrine systems, to elucidate possible relationships with behaviour, and to link rodent data with exposures and effects observed in humans.

Acknowledgments: This project has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No. 825759 (ENDpoiNTs).

References:
MON-PM1-E2  Structure of the dioxin receptor (AHR) revealed by cryo-electron microscopy

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Introduction: Living organisms have developed protein sensors that help them adapt to their environment. The aromatic hydrocarbon receptor (AHR) is an emblematic member of this class of proteins. AHR is a ligand-dependent transcription factor involved in a wide range of physiological and pathological processes in response to hundreds of chemicals or natural substances. Notably, AHR is historically known as the receptor for many pollutants such as polycyclic aromatic hydrocarbons (PAHs), halogenated aromatic hydrocarbons (HAHs) or polychlorinated biphenyls (PCBs), mediating both their metabolism and deleterious effects. Until now, the high instability of this protein and the resulting lack of high-resolution structural data limited our understanding of the molecular mechanisms by which AHR activity can be modulated by such a diversity of compounds.

Materials and Methods: Using the SF9 insect cell expression system, we reconstituted the cytosolic AHR complex by co-expressing a fragment of human AHR (residues 1-437) in the presence of the chaperone Hsp90 and co-chaperone XAP2. The Hsp90-XAP2-AHR complex was purified in the presence of indirubin, a natural ligand of AHR, and the structure was solved by cryo-electron microscopy (cryo-EM). The structure was further analyzed using structure-guided mutational analysis and molecular dynamics (MD) simulations.

Results: The structure at a resolution of 2.8 Å reveals a concerted action of Hsp90 and XAP2 to maintain the receptor in a stable and functional form as confirmed by mutational analyses. Importantly, it provides the first experimental visualization of the ligand binding domain of AHR and reveals a unique organization of the pocket where the small regulatory molecules bind. More specifically, the structure allows us to explain the mechanism behind promiscuity and selectivity of the receptor towards compounds exhibiting a planar shape (e.g. dioxin), and MD simulations provided insight into the potential entry site of the ligand.

Discussion and Conclusion: By revealing the molecular determinants of ligand-binding specificity and promiscuity of the receptor, our study rationalizes more than forty years of biochemical data and sheds new light on the function of this important receptor for the regulation of the interactions between the host and its environment. Furthermore, this atomic-scale information provides a rational framework for better understanding the impact of environmental pollutants on human health. Structural analyses are ongoing in our lab to better characterize the interaction between AHR and prototypical environmental ligands (PAHs, HAHs and PCBs).

Acknowledgments: The work was supported by funding from European Union’s Horizon 2020 research and innovation program grant GOLIATH No. 825489, ATIP-Avenir 2020 grant No. R20059SP and the ANR (French National Research Agency) “Investissements d’avenir” programme reference No. ANR-16-IDEX0006.

MON-PM1-E3  Autistic-like behaviour and immune dysfunction in rats early-exposed to α-HexaBromoCycloDecane: A potential role of this brominated flame retardant in the emergence of Autism Spectrum Disorders

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Introduction: Early-life exposure to α-hexabromocyclododecane (α-HBCDD), a brominated flame retardant, is suspected to contribute to the increased prevalence of Autism Spectrum Disorders (ASD). Previous research has demonstrated that α-HBCDD induces behavioural impairments similar to those observed in the valproate (VPA) rodent model of ASD during the early stages of development (PND2-21). The objectives were then to evaluate the effects of such exposure later in life and confirm the induction of a genuine ASD phenotype.

Material and Methods: Pregnant Wistar rats were divided into three groups (n=6 dams per group): control, α-HBCDD (100 ng/kg/day p.o., GD0-PND21), and VPA (500 mg/kg i.p., GD12). The male offspring’s autistic phenotype was assessed at PND50 using four main behavioural criteria: lack of social interactions, stereotypies, sensory impairments, and hyperactivity/anxiety. At the end of the experimental design, blood (100µL per animal) was collected to perform immunophenotyping by flow cytometry. The brain regions were microdissected to assess the neuroinflammation by cytokine array in the cortex.

Results and Discussion: The results of the behavioural analysis revealed a significant decrease in sociability in males exposed to α-HBCDD or VPA compared to controls in the 3-chamber test. Indeed, no significant difference were noted in the time spent exploring a familiar and unfamiliar congener for the exposed groups. An increase in stereotypies, including a higher number of marbles buried and circling behaviour, was observed in the α-HBCDD and VPA groups compared to controls (p<0.01). Both exposed groups also showed altered thermal and mechanical pressure sensitivity (p<0.05 and p<0.01, respectively) compared to controls. Taken together, these findings indicate behavioural abnormalities associated with ASD-like symptoms. Moreover, α-HBCDD exposure triggered immune system abnormalities, similar to VPA, as evidenced by changes in the immunophenotype of two cell populations (a decrease in 16.1% in Natural Killer cells and 58.8% in Tc1 cells). This suggests a potential immunosuppression and/or immune cell infiltration. In parallel, specific cortical α-HBCDD- and VPA-related changes in the expression of 26 proteins were observed, involved in the PI3K/AKT/mTOR and JAK/STAT pathways. Dysregulation of these pathways has also been implicated in ASD patients.

Conclusions: This study highlights the neurotoxic and immunotoxic effects of α-HBCDD, comparable to the effects of VPA, suggesting the potential involvement of α-HBCDD in the emergence of ASD. Ongoing global gene expression analysis aims to provide further insights into the underlying mechanisms linking early-life α-HBCDD exposure to the development of ASD. By understanding these mechanisms, we can gain a better understanding of the impact of environmental factors on ASD and contribute to the development of preventive measures in general populations.

Acknowledgments: The authors would like to express their gratitude to A2F young research grant (2019-2020). We acknowledge the support of the “Ministère de l’Enseignement supérieur et de la Recherche” in France. This study was partly funded by the Fonds National de Recherche Luxembourg: FNR-CORE (C16/BM/11342695 "MetCOEPs") and FNR INTER (INTER/ANR/16/11568350 “MADAM”).
MON-PM1-E4  Importance of PCB Chiral Separation and Structural Prediction

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Introduction: The ryanodine receptor regulates intracellular calcium levels and plays a crucial role in brain function. It has
been extensively studied in synaptic plasticity, neuronal excitability, and excitatory neuronal death. The receptor is named after
its interaction with ryanodine, a plant compound, and exists in three distinct subtypes.

Püttmann1) synthesized and measured the optical rotation of PCB atropisomers (PCB-88, 139, 197), distinguishing between
(-) and (+) configurations using pure samples. Additionally, Haglund2) separated and determined the optical rotations of nine
PCB atropisomers (PCB-84, 131, 132, 135, 136, 174, 175, 176, 196) using RP-HPLC. We developed a rapid method for chiral
separation and identification3) of PCB atropisomers, applied to PCB metabolism and enantioselective RyR studies. 4–7)

Materials and Methods: HPLC -UV (Tosoh CO-8020, Shimadzu LC-10AT) was used for chiral separation with specific parameters.
Chiral separation of PCB atropisomers was performed using a Daicel Chiralcel OJ-H column. We optimized separation conditions
by adjusting column temperature, flow rate, elution solvent, injection volume, and sample concentration. PCB samples were
sourced from Accu Standard, and UV detection at 291 nm was utilized. For PCB-183 analysis in biological samples, GC/HRMS
was employed. To calculate specific rotation and assess chlorine substitution positions in PCBs, we used the def2-TZVP basis
set and B3LYP density functional theory (DFT). Optical rotation calculations were conducted using Turbomole software.

Results: The rapid method for chiral separation and identification of PCB atropisomers is a significant breakthrough in
PCB metabolism and enantioselective RyR research. High-purity samples and RP-HPLC effectively distinguish (-) and (+)
configurations. DFT calculations evaluate chlorine substitution positions and optical rotations. Integration of experimental and
theoretical data provides strong evidence for method accuracy. Agreement between circular dichroism spectra and optical
rotations further demonstrates effectiveness. This research facilitates future investigations and improves understanding of
PCBs’ environmental and health effects. Optical rotation measurements using short columns and low resolution (R) validate
the method. Metabolic differences emphasize the need to assess individual biological risks. The method successfully separates and identifies challenging isomers through optical rotation data.

Discussion and Conclusion: The developed method is a significant breakthrough, rapidly separating and identifying PCB
atropisomers. It ensures accurate results with high-purity samples and RP-HPLC. DFT calculations enhance understanding
of chlorine substitution positions and optical rotations. Agreement between circular dichroism spectra and optical rotations
supports reliability. Collaboration validates optical rotation measurements with short columns and low resolution (R). Metabolic
differences highlight the importance of assessing individual risks. The method’s implications extend to PCB metabolism and
enantioselective RyR studies. It contributes to understanding PCBs’ environmental and health effects. Overall, the developed
method has significant implications for PCB metabolism and enantioselective RyR studies. It not only facilitates future
investigations in these fields but also contributes to a better understanding of the environmental and health effects associated
with PCBs. The method’s speed, accuracy, and ability to distinguish between different atropisomers make it a valuable tool
for researchers in various applications related to PCB analysis. Among the 19 chiral PCBs examined, four isomers (PCB95,
PCB144, PCB149, and PCB183) with substitutions at positions 2, 4, or 2, 4, 5 exhibited the aR-(+) and aS-(+) configuration, while
the remaining 15 types displayed the aR-(-) and aS-(+) configurations.

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MON-PM1-E5 Developmental Effects of 2,3,7,8-Substituted and Non-2,3,7,8-substituted Polybrominated Dibenzo-p-dioxins in Zebrafish

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Introduction: Polybrominated dibenzo-p-dioxins (PBDDs) have been detected in marine organisms. For instance, 1,3,7-TrBDD and 1,3,8-TrBDD are the most abundant PBDDs in blue mussels and sponges in the Baltic Sea1,2. 2,3,7,8-Substituted PBDDs can bind to the aryl hydrocarbon receptor (AHR), resulting in a variety of toxicity. Studies on the developmental effects of PBDDs in fish are available mainly on 2,3,7,8-substituted PBDDs, showing 2,3,7,8-TeBDD is the most toxic congener3. However, developmental effects and their mechanisms induced by non-2,3,7,8-substituted PBDDs in fish are still limited. The present study assessed developmental effects caused by three non-2,3,7,8-substituted PBDDs, 1,3,7-TrBDD, 1,3,8-TrBDD, and 2,3,7-TrBDD, as compared to 2,3,7,8-TeBDD, using zebrafish as a model species.

Materials and Methods: Zebrafish embryos were exposed to DMSO or different concentrations of 1,3,7-TrBDD, 1,3,8-TrBDD, 2,3,7-TrBDD, or 2,3,7,8-TeBDD, beginning at 24 hours post fertilization (hpf). Co-exposure to those PBDDs and an AHR antagonist CH-223191 (CH) was also performed. At 96 hpf, embryos exposed to each of PBDDs with or without CH were examined for pericardial edema and blood flow reduction or for measurement of CYP1A mRNA expression level. For RNA-Seq, embryos exposed to DMSO, 1,3,7-TrBDD, 1,3,8-TrBDD, or 2,3,7,8-TeBDD were sampled in a separate experiment. Genes with |log2 fold changes (log2 FC)| > 1 and false discovery rate (FDR) < 0.05 were considered as differentially expressed genes (DEGs).

Results: All tested non-2,3,7,8-substituted PBDDs and 2,3,7,8-TeBDD induced CYP1A expression in a concentration-dependent manner. The EC50-based rank order of CYP1A induction was 2,3,7,8-TeBDD (0.02 ppb) > 2,3,7-TrBDD (0.59 ppb) > 1,3,8-TrBDD (10 ppb) ≈ 1,3,7-TrBDD (25 ppb). The 2,3,7,8-TeBDD-induced CYP1A expression was largely inhibited by co-exposure to the AHR antagonist. Regarding the non-2,3,7,8-substituted PBDDs, the AHR antagonist clearly inhibited the CYP1A induction by 2,3,7-TrBDD. It suppressed the 1,3,7-TrBDD-induced CYP1A expression only partially, whereas it failed to inhibit the CYP1A expression induced by 1,3,8-TrBDD. A concentration-dependent increase in pericardial edema and blood flow reduction was observed in the three tested chemicals, showing the highest potency seen in 2,3,7,8-TeBDD, followed by 2,3,7-TrBDD and 1,3,7-TrBDD. No major developmental toxicity was elicited by 1,3,8-TrBDD with the concentrations up to 100 ppb. The AHR antagonist significantly rescued the circulatory failure induced by 2,3,7-TrBDD and 2,3,7,8-TeBDD. RNA-Seq analysis showed that genes involved in metabolism, homeostasis, and transport of lipids and innate immunity were enriched by the 2,3,7,8-TeBDD exposure. A number of genes in the solute carrier family were included as DEGs in the lipid-related pathways, while chemokine and complement genes were mainly detected as DEGs in the innate immunity pathways. The 1,3,7- and 1,3,8-TrBDDs exposure, as well as 2,3,7,8-TeBDD exposure, elicited significant DEGs in the pathway related to the circadian clock.

Discussion and Conclusion: The present study clearly showed that 2,3,7-TrBDD exhibited CYP1A induction and developmental anomalies through the AHR. 1,3,7-TrBDD induced CYP1A expression partially through the AHR, while 1,3,8-TrBDD induced CYP1A expression via the AHR-independent pathway. Therefore, it is shown that the number and position of bromine substitutions at the lateral position of TrBDDs seem to be important for the potency of the AHR-mediated effects. It has been reported that AHR agonists may disrupt energy metabolism and immune responses through altered circadian rhythm4-6. Thus, the current RNA-Seq results indicate that the disruption of the circadian signaling followed by an altered metabolism of glucose and lipids might be involved in the cardiovascular toxicity caused by non-2,3,7,8-substituted TrBDDs, as well as 2,3,7,8-TeBDD.

Acknowledgments: This research was supported by the Grant-in-Aid for Young Scientists (A) (15H05334) and Grant-in-Aid for Scientific Research (B) (19H04275 and 22H03746) from JSPS.

References:
Per- and Polyfluorinated Substances (PFAS): Occurrence and Exposure

**S. Brandsma & Y. Fujii**

MON-PM2-A1 PFAS or SeaFAS: Seafoam as a potentially significant PFAS carrier

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**Introduction:** Per- and polyfluoralkyl substances or PFAS or synthetic organofluorine chemicals. The chemical family contains over 6000 PFAS are widely used as they have unique desirable properties. They are stable under intense heat, many of them are also surfactants and are used as water and grease repellents. Scientists and governments around the world have recognized the harmful effects of PFAS on human health and the environment. Global occurrence and distribution of PFAS has been the subject of research for many decades, however it is not yet well understood. Recently, a Great Lakes study focused on PFAS present in foam present on the water surface (Schwichtenberg et al., 2020). A study by Sha et al. raised attention to the presence of PFAS in airborne coastal aerosols (Sha et al., 2022). This raised concern and the hypothesis that PFAS would also be present in seafoam formed by waves in combination with currents and wind. Seafoam is a naturally occurring phenomenon. Seawater contains dissolved salts, proteins, fats, dead algae, detergents and a wide array of (anthropogenic) pollutants. When powerful waves and tides have free play sea foam is formed on the water surface and the foam is left behind when it lashes the shores.

**Materials and Methods:** Seafoam (n = 7) and seawater (n = 2) were sampled at 2 independent and random locations along the Belgian shoreline (Knokke and De Haan) in 2021 and 2022. One additional seafoam sample was collected at the French coast near Boulogne-sur-mer, which approximately 100 km south of the main sampling locations in Belgium. Foam samples were left standing until the foam collapsed. Samples were further treated as water samples and analysed using the Flemish official PFAS method for water samples (WAC/IV/A/025). In addition, sand from the same locations was also sampled (n = 5). Sand samples were dried and analysed using the Flemish official PFAS method for solid samples (CMA/3/D). These methods have a PFAS-scope of around 40 different compounds, including branched and linear isomers.

**Results:** The PFAS concentrations in the condensed seafoam ranged from 8.7 µg/L to 2,400 µg/L for the sum of all measured PFAS. The sum of the 4 EFSA compounds (PFOS, PFOA, PFNA en PFHxS) contriuted to 90 % of the total PFAS load. PFAS levels in the seawater were lower than 10 ng/L, which is the LOQ of the WAC-method. PFAS could however be detected at levels around 1-3 ng/L. An exception was noted for PFBA, which was present at 250 ng/L in a water sample from De Haan. The seafoam sample from Boulogne-sur-mer contained a 170 µg/L for the sum of all PFAS measured. The total PFAS concentration in the soil/sand samples ranged from 0.06 tot 10 µg/Kg dm.

**Discussion and Conclusion:**

The different Belgian and French seafoam samples contained comparable amounts of PFAS, although the samples were not linked, nor in space, not in time. This indicates that the phenomenon is widespread and the concentration range that was recorded is consistent. Levels in seawater were significantly lower than in foam, confirming the hypothesis that PFAS enriches during the natural foam forming process, also in sea water. After enrichment foam can be spread by wind and broken down to form aerosols, which in turn is a potential source of PFAS distribution in coastal areas. This was already noted by Sha et al. (2022). In addition to Levels in sand taken from the shoreline were significantly lower than in sand taken from dunes that are located in the dry zone of the beach. Potential PFAS enrichment in sandy dunes through airborne foam and aerosols is considered a potential explanation for this observation.

The current study was not more than a feasibility study to test the hypothesis of PFAS seafoam accumulation. This hypothesis was confirmed and merits further research. We ask for European partnerships to take this seafoam and coastal distribution research to the next level by means of a European wide collaboration encompassing the complete European coastline.

**Acknowledgments:** This study was commissioned by the Flemish Agency for Care and Health (Vlaams Agentschap Zorg en Gezondheid (VAZG). The authors acknowledge Barbara Legiest and Bart Bautmans for their interest in the topic and providing funding.

**References:**

Per- and Polyfluorinated Substances (PFAS): Occurrence and Exposure
S.Brandsma & Y.Fujii

MON-PM2-A2  TFA and other PFAS in rainwater from the Norwegian Arctic and mainland

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Introduction:
Several anthropogenic sources of trifluoroacetic acid (TFA) have been identified. These sources include direct uses by industry (1), biodegradation of fluorochemical precursors, pesticides and pharmaceuticals that contain a –CF₃ group, and the atmospheric photochemical oxidation of certain F-gases that were introduced as replacements to chlorofluorocarbons after the Montreal protocol (2-5). TFA and other small fluorinated acids are highly water soluble and hardly bioaccumulative. In our study we aimed at investigating the presence of small fluorinated acids in rainwater collected in the Norwegian Arctic (Ny Ålesund, Svalbard) in comparison with reference sites on the Norwegian mainland (Birkenes) and an urban setting (City of Trondheim), a coastal site (Veiholmen) and a popular recreational area (Selbu).

Materials and Methods:
Sampling duration was 14 days at each sampling site over the period of 6 months in 2022. The water was collected and stored in precleaned glass bottles, transported to the lab and filtrated under clean room conditions. LC-MS analyses of sample extracts were carried out.

Results:
TFA and other PFAS were found in most of the samples. TFA results are shown in Figure 1. The most remote location at the Zeppelin station, Ny Ålesund, Svalbard, showed the lowest concentration over the sampling period, however, episodes of elevated TFA deposition could be found. Of the other detected PFAS, PFOA was among the most commonly found PFAS.

Discussion and Conclusion:
The ubiquitous detection of TFA and legacy PFAS in rainwater proves their global distribution and accumulation. Further clouds and rain are an efficient transport route of PFAS to reach remote locations. The concentrations in Trondheim being similar to the remote arctic concentrations show that the levels observed could serve as the current global background level.

References:
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5. Holland et al., 2021
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1. Introduction:
Per- and polyfluoroalkyl substances (PFAS) is a group of anthropogenic fluorinated chemicals which contain a perfluorinated methylene (-CF2-) or perfluorinated methyl group (-CF3) according to a new definition (OECD 2021). PFAS are extensively used in diverse fields and some legacy PFAS (perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid (PFOS) and perfluorohexane sulfonic acid (PFHxS)) have already been regulated under the Stockholm Convention due to their increasing environmental concerns. The regulations have led to the phase-out of these substances and manufacturers have shifted to the compensatory production and usage of replacements. However, current targeted methods showed limitations in comprehensively characterizing PFAS especially the replacement products since their chemical identities are not known. Hence, extractable organofluorine (EOF)-based mass balance analysis has been used to obtain information overlooked from targeted analysis (Aro et al. 2021). A recent study reported the detection of tetrafluoroborate (BF₄⁻) and hexafluorophosphate (PF₆⁻) in German river systems (Neuwald et al. 2021). These compounds might have been co-extracted during EOF analysis. Therefore, “extractable fluorine (EF) mass balance and unexplained extractable fluorine (UEF)” are more appropriate compared to EOF.

Previous study has identified drinking water as an important exposure way to human. Therefore, tap water samples were collected from China (one city), Sweden (three cities), Norway (two cities) and Denmark (one city) to conduct EF mass balance analysis and to evaluate the role of BF₄⁻ and PF₆⁻ in closing mass balance.

2. Materials and Methods:
2.1 Chemicals
A total number of 35 PFAS was the target compounds. Analytical standards including perfluoroalkyl carboxylic acids (PFCAs, C4-C14, C16 and C18), perfluoroalkyl sulfonic acids (PFSAs, C3-C10 and C12), fluorotelomer sulfonic acids (FTSAs, 4:2, 6:2 and 8:2), chlorinated perfluorinated ether sulfonic acids (6:2 Cl-PFESA, 8:2 Cl-PFESA), perfluoroethylcyclohexane sulfonic acid (PFECHS), perfluorooctane sulfonamide (FOSA) were purchased from Wellington Laboratories (Guelph, ON, Canada). Trifluoroacetic acid (TFA), perfluoropropanoic acid (PFPrA) and trifluoromethane sulfonic acid (TFMS) were purchased from Sigma-Aldrich. Perfluoroethane sulfonic acid (PFEtS) was purchased from Kanto Chemicals Xo., Inc.

2.2 Sample collection and pretreatment
2 L of tap water samples were collected from China (Shanghai, n=39), Sweden (n=3), Denmark (n=1) and Norway (n=2). All samples were stored in polypropylene (PP) bottle and kept at 4°C until analysis. Solid phase extraction (SPE) method was performed to extract PFAS in tap water samples. Samples were extracted in duplicate; subsample of 500 mL was used for target analysis, whereas 1 L for EF analysis were extracted with Oasis WAX cartridges (Waters 150 mg, 6 mL, 30 µm). For target analysis, mass labelled internal standards were spiked before extraction. Analytes were eluted with 4 mL of methanol with 0.1% NH₄OH and then concentrated under nitrogen, and mass labelled recovery standards were added for further splitting for analysis. For EF analysis, neither mass labelled internal standard nor recovery standards were added. The procedures were similar to the target analysis except with the additional washing with 0.01% NH₄OH in water to removal any inorganic fluoride that might have enriched during extraction.

2.3 Oxidative conversion
The oxidative conversion was modified based on the previous method (Houtz and Sedlak 2012) and was performed on sample extracts to estimate the occurrence of unknown precursors. The extracts were dried to near dryness and amended with 0.75 mL of 10 M NaOH solution and 25 mL of 120 mM K₂S₂O₈ solution. Samples were then placed into the water bath (85°C) for 6 h. After the reaction, samples were cooled down to room temperature and pH was modified to 2 with formic acid. Extractions followed similar procedure described in section 2.3 but with a larger amount of sorbent material and larger particle size (500 mg, 60 µm).
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2.4 Suspect screening
Suspect screening analysis was performed by an UPLC system coupled to a quadrupole time-of-flight mass spectrometer (G2-XS) in the electrospray negative ionization (ESI-) mode using an Acquity BEH C18 column (2.1 mm x 100 mm; 1.7 µm). A data independent acquisition mode (MSE) was used to obtain the precursor and fragment ions. Details were provided elsewhere (Koch et al. 2021).

2.5 Instrumental analysis for target PFAS and EF
Ultra-short PFAS, BF$_4^-$ and PF$_6^-$ were analyzed using a supercritical fluid chromatograph (SFC) with a Torous DIOL column (3 mm x 150 mm, 1.7 µm) coupled to a tandem mass spectrometer (MS/MS) (Xevo TQ-S) in electrospray negative ionization (ESI-) mode. The analysis of other target PFAS were conducted on ultra-performance liquid chromatography electrospray ionization tandem mass spectrometry (UPLC-MS/MS) in ESI- mode with a BEH C18 column (2.1 mm x 100 mm, 1.7 µm). The gradient conditions were applied and more details are provided elsewhere (Björnsdotter et al. 2019).

EF was analyzed by combustion ion chromatograph (CIC). In brief, the sample (100 µL) was placed on a quartz boat and all fluorine was converted to hydrogen fluorine and adsorbed into the water after combusting at 1000–1050 °C. The dissolved fluoride was then analyzed by the ion chromatograph. Separation of anions was completed by an ion exchange column (Metrosep A Supp5, 4 mm x 150 mm) with 64 mM sodium carbonate and 20 mM sodium bicarbonate in water as the eluent solution. Details of the method are provided in previous paper (Aro et al. 2021).

2.6 Quality assurance and quality control
For target analysis, two procedure blank samples and one recovery sample (with native standards spiked) were extracted for every batch. A homogeneously mixed sample was prepared by mixing all the water samples and then split into subsamples and was extracted to evaluate reproducibility of extractions for every batch for EOF analysis. An internal calibration method was used for quantification. The method detection limit (MDL) was determined as average concentrations in procedural blanks plus three times the standard deviation and method quantification limit (MQL) was determined as average plus ten times the standard deviation. The lowest calibration curve point was used as MQL if the compound was not detected in blanks. EF was quantified using an external calibration curve using PFOA as analytical standard (ranging from 50-1000 ng /L F equivalents).

3. Results:
Trace levels of organofluorine contamination were detected in extraction blank (< 50 ng/L) and trace detectable PFAS levels were found in extraction blank (<0.3 ng/L). Recovery of target PFAS ranged from 40 to 110%. Since there is no certified materials for EOF analysis, the homogeneously mixed sample was used to assess the reproducibility of the method; the relative standard deviation among different extraction batch was found to be less than 40%.

3.1 The occurrence of target PFAS
The total PFAS concentrations in tap water ranged from 70 (Denmark) to 3300 (China) ng F/L. The PFAS profiles are shown in Figure 1. Across all samples, the largest contributor to PFAS was TFA, accounting for more than 90% of PFAS. The remaining PFAS (PFCAs, PFSA and some novel PFAS) made a minor contribution (less than 2% of PFAS). Of the remaining PFAS, PFCAs contributed a further less than 1.5% of the PFAS. The mostly detected PFCAs and PFSA are PFBA, and PFBS and PFHxS, respectively.

Figure 1: Profiles of target PFAS in tap water samples from China, Sweden, Norway and Denmark (%).
3.2 EF mass balance analysis
Since BF$_4^-$ and PF$_6^-$ were detected in the fraction for EOF analysis, the term extractable fluorine is used here. Before conducting mass balance analysis, measured concentrations of target PFAS, BF$_4^-$ and PF$_6^-$ need to be converted into fluorine-equivalent concentration. Similar calculations are provided in a previous paper (Miyake et al. 2007). EF mass balance of tap water samples from different countries are presented in Figure 2. The EF concentrations were in the range of 83 to 420 ng F/L. Mass balance could be closed in samples from Norway. The great proportion of UEF was found in samples from China (75%), Sweden (55%) and Denmark (90%). The identified EOF fraction was driven by ultra-short PFAS which accounted for 10-97% of EF. In samples from China, less than 30% of EF was UEF when BF$_4^-$ and PF$_6^-$ were taken into account and mass balance could even be closed in some samples. BF$_4^-$ and PF$_6^-$ were detected in tap water samples from China at the concentrations of 70 and 180 ng F/L, respectively and showed no detection in samples from Sweden, Norway and Denmark.

3.3 Oxidative conversion and suspect screening results
In tap water from China, an additional eight PFAS were identified through suspecting screening. Sodium p-perfluorous nonenoxybenzenesulfonate (OBS) and bis(trifluoromethanesulfonyl)imide (NTf$_2^-$) were detected in all of the samples. However, total semi-quantification concentrations of all the novel PFAS were less than 1 ng/L which didn’t contribute a lot to missing EF. Oxidative conversion results showed that unknown oxidizable precursors did not make significant contributions to UEF with the increasing ratios of individual PFAS less than 30% after reactions. No novel PFAS were identified through suspecting screening in tap water from Sweden, Norway and Denmark.

4. Discussion:
For tap water from China (Shanghai), EOF mass balance analysis was conducted in the beginning and target PFAS only explained less than 40% of EOF. A large fraction of EOF remained unknown although suspect screening identified few compounds. However, the detection of BF$_4^-$ and PF$_6^-$ indicated that inorganic fluorinated ions might also exist in final sample extracts. Therefore, EF mass balance is more appropriate. After taking BF$_4^-$ and PF$_6^-$ into account, EF mass balance could be closed for some of the samples and UEF were largely reduced (<30%). The two compounds seems to be source specific since no detection was found in samples from other countries and they were found to be related to ionic liquids and electrolyte salts which was similar to NTf$_2^-$ (Lu et al. 2006; Neuwald et al. 2021).

For tap water from Norway, EF mass balance was closed and TFA was the largest contributor which accounted for more than 95%. For tap water samples from Sweden and Denmark, the proportion of UEF was more than 50%. Suspect screening was also conducted to identify what compounds might contribute to UEF. However, no novel PFAS were identified. In addition to TFA, BF$_4^-$ and PF$_6^-$, other very mobile fluorinated compounds which are difficult to analyze on normal C18 column could also be potential sources. Fluorinated pharmaceuticals might help explain UEF as well. There, these possible substances need to be further investigated.

However, the current EF mass balance analysis still suffers from the limitation of balancing the retention of TFA with removing inorganic fluoride. In addition to inorganic fluoride, our unpublished data also showed the retention of BF$_4^-$ and PF$_6^-$ after performing washing step during extractions which require further investigation. Despite the limitations, EF mass balance analysis remains a useful method in estimating “total PFAS” and the profiles in “total PFAS”. The combination of EF and target PFAS in EF extracts can provide a more comprehensive assessment and can help track the compounds in EF fractions.
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5. Conclusions:
In this study, EF mass balance analysis was conducted in tap water samples from China, Sweden, Norway and Denmark. Notably, BF$_4^-$ and PF$_6^-$ showed detection in tap water from China, UEF was below 30% and mass balance was even closed in some samples. However, BF$_4^-$ and PF$_6^-$ seems to be source specific since no detection was found in other samples. Therefore, further research should pay more attention to these inorganic fluorinated anions and counter cations to better understand their source and occurrence. Further efforts will also be required to better understand the occurrence of other inorganic fluorinated anions (if any) and therefore, a more comprehensive EF mass balance analysis can be obtained.

6. Acknowledgments:
The study was financially supported by the National Key Research and Development Project of China (2021YFC3200801). We also acknowledged funding from the Knowledge Foundation (KKS) within the Enforce Research Profile (20160019), Sweden, and Swedish Research Council FORMAS (2020-02032) and grant from Eurofins Environment Testing Sweden AB.

7. References:
Mon-PM2-A4 PFAS in Selected Products in Indonesia

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Abbreviations
PFBA Perfluorobutyric acid
PFDA Perfluorodecanoic acid
PFDoA Perfluorododecanoic acid
PFHpA Perfluoroheptanoic acid
PFHxA Perfluorohexanoic acid
PFNA Perfluorononanoic acid
PFOA Perfluorooctanoic acid
PFUdA Perfluoroundecanoic acid
PFOS Perfluorooctane sulfonate
PFPeA Perfluoropentanoic acid
PFTeDA Perfluorotetradecanoic acid
PFTrDA Perfluorotridecanoic acid
PFHxDA Perfluorohexadecanoic acid
PFODA Perfluorooctadecanoic acid
FTS Fluorotelomer sulfonate
FTOH Fluorotelomer alcohol
monoPAP Fluorotelomer phosphate monoester
diPAP Fluorotelomer phosphate diester

1. Introduction:
Per- and polyfluoroalkyl substances (PFAS) or "Forever Chemicals" are a large class of synthetic substances that are widely distributed in the global environment due to their high solubility in water and low/moderate sorption to soils and sediments as well as high resistance to biological and chemical degradation. Common sources of human exposure to PFAS substances are food, water, air, and dust. These substances bind to proteins – not to fats – and persist in the body, where they are mainly detected in blood, liver, breastmilk, and kidneys. Exposure to PFAS poses a health risk not only for humans, but also for wildlife animals. Forever Chemicals continue to be detected in aquatic biota across the globe including Arctics. Recent studies have linked a variety of PFAS substances to many human health effects: cardiovascular disease, markers of asthma, damage to semen quality, ovarian insufficiency, altered glucose metabolism, lower testosterone levels in male adolescents, association with shorter birth length in girls, elevated blood pressure, abnormal menstruation, lower birth weight in infants, possible increased risk of female infertility due to endometriosis, and decreased lung function in children with asthma.

PFAS are used by many industries, including aerospace, construction, automotive, textiles, paper and pulp and electronics, because of their ability to reduce friction on surfaces and provide grease- and water-resistance. A presence of various PFAS-treated products at home contributes to human exposures via inhalation of house dust or during skin contact with the products itself. PFAS from a variety of sources end up on the skin, including the hands. PFAS levels on the skin have been correlated with PFAS in house dust and PFAS precursors in indoor air. Many PFAS-treated products end up in landfills or are incinerated. Disposal of end-of-life products in municipal incinerators leads to emissions of PFAS, fluorinated greenhouse gases and other products of incomplete combustion to the surrounding environment. Some PFAS remain in the after-incineration fly ash, and then contribute to the further environmental exposures when the fly ash is landfilled or used in construction materials. The recycling of PFAS-treated consumer products leads not only to exposure of consumers, but also of workers and communities living nearby recycling plants. Workers can be exposed to PFAS when waste material is shredded and ground, and surrounding communities are exposed when PFAS are emitted into the water.

This study was conducted to assess PFAS utilization in selected products in Indonesia. It aims to contribute to the discussion on the integrity of a non-toxic circular economy and on the universal ban of Forever Chemicals.
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2. Materials and Methods:
Nexus3 and IPEN collected 48 samples from Indonesia and the U.S. with potential PFAS treatment or contamination including clothing/apparel products, microwave popcorn bags, paper food packaging, thermal paper and one sample of rubber crumbs. The samples were collected from 2019 to 2022. Thirty to fifty-six PFAS congeners were analyzed in the collected samples.

<table>
<thead>
<tr>
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<th>Recycled rubber crumbs</th>
<th>Textile, apparel</th>
<th>Food contact materials</th>
<th>Thermal paper</th>
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<td>Paper popcorn bags</td>
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<td>contact materials</td>
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<td>2019</td>
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<td>1</td>
<td>3</td>
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<td>2020</td>
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<td>5</td>
<td>10 Indonesia</td>
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<td>7 Indonesia</td>
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All samples were analyzed at the University of Chemistry and Technology in Prague, Czechia using ultra-high performance liquid chromatography interfaced with tandem mass spectrometry with electrospray ionization in negative mode (UHPLC-MS/MS-ESI-). For isolation of selected per- and polyfluoroalkylated substances (PFAS), ultrasound-assisted extraction using a mixture of methanol:ethyl acetate (1:1, v/v) was applied.

Expanded uncertainty was calculated using coverage factor k=2, corresponding to a coverage probability of approximately 95%. The lab followed the EA-4/16 and manual Kvalimetríe 11 (issued by EURACHEM CZ) to calculate and state uncertainty. Uncertainty of sampling is not covered. Compliance is evaluated with respect to the uncertainty of test results according to the Guide ILAC-G8.

3. Results:
Almost all the tested samples (93.7%) contained at least one PFAS congener above the limit of quantification (LOQ) (See Figure 1). The highest levels of PFAS among analyzed samples were present in a microwave popcorn bag. The microwave popcorn products with the highest PFAS concentrations contained 2,043 ng/g of 6:2 diPAP (in Preferred Kettle Corn popcorn bag purchased in Indonesia) and 730 ng/g of 6:2 FTOH (in Jolly Time Blast O Butter microwave popcorn purchased in the U.S.).
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**Figure 2:** Frequency of PFAS congeners in samples of different groups

**Figure 3:** PFAS concentration distribution in popcorn bag samples from Indonesia and the U.S. in ng/g. One sample is excluded from this figure due to high concentration (2,043 ng/g)

**4. Discussion:**

**PFAS in microwave popcorn bags and paper packaging**

In accordance with our findings, DiPAPs, FTOHs and PFCAs are typical PFAS chemicals identified by different researchers in food packaging from various countries.

PFAS in microwave popcorn bags can migrate into the oil, making it available for ingestion. This can add to existing exposure to polluted food and water. A study in 2019 found that people who regularly ate microwave popcorn tended to have significantly higher PFAS levels in their blood.

The presence of PFAS in food packaging raises concerns as migration of PFAS from food contact materials into food may also contribute to a major route of PFAS exposure via diet.
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PFAS in textile products
The hijab and the children’s t-shirt in this study exceeded the weak EU regulatory limit set in POPs regulation of 25 ppm (ng/g) PFOA. The level of PFOA in the children’s T-shirts was 1.8 times higher than the EU regulatory limit (25 ppm or ng/g).

Laboratory weathering of textiles containing PFAS to mimic the lifespan of outdoor clothing resulted in significant releases of PFAS substances. Moreover, several studies revealed that PFAS can penetrate human and mouse skin, therefore researchers expressed concern about potential dermal exposure to PFAS in both occupationally exposed individuals and the general population.

The use of PFAS in products for children and women accompanied with the fact that PFAS can weather from the textile and contribute to dermal exposure raises serious concerns, as PFAS are known to be endocrine disrupting chemicals (EDCs) that negatively affect thyroid hormones.

PFAS in rubber crumbs
Findings of PFAS in rubber crumbs that is used in artificial turf have been found in other studies. The crumbled rubber pellets can get into shoes or clothing and end up in cars and homes. The utilization of PFAS-rich rubber crumb in artificial turf can lead to additional exposure to children and young sports players in Indonesia.

Potential impacts of single-use packaging and other PFAS-treated products at the end of life
Many PFAS-treated products end up in landfills or are incinerated. Disposal of end-of-life products in municipal incinerators leads to emissions of PFAS, fluorinated greenhouse gases and other products of incomplete combustion to the surrounding environment. Some PFAS remain in the after-incineration ash, and then may contribute to the further environmental exposures when the ash is landfilled or used in construction materials. The recycling of PFAS-treated consumer products leads not only to exposure of consumers, but also of workers and communities living nearby recycling plants. Workers can be exposed to PFAS when waste material is shredded and ground, and surrounding communities are exposed when PFAS are emitted into the water.

5. Conclusions:

- 22 PFAS congeners (PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUdA, PFDoA, PFTdA, PFTeDA, PFHxDA, PFODA, 6:2 FTOH (FHET), 8:2 FTOH, 12:2 FTOH, 6:2 diPAP, 8:2 diPAP, 6:2 8:2 diPAP, 6:2 FTS, 6:2 monoPAP and 8:2 monoPAP) were identified in analyzed items.
- The PFAS with the highest concentration (30,178 ng/g) in textile was 6:2 diPAP, found in a water-proof hijab purchased in Indonesia.
- The PFAS with the highest concentration (2,043 ng/g) in food packaging paper was 6:2 diPAP, found in a microwave popcorn bag imported from the U.S.
- The most frequently detected PFAS chemicals were 6:2 FTOH, PFHxA, PFBA, PFHpA, PFOA, PFNA, PFDA, PFUdA, PFDoA, PFTdA, PFTeDA, PFHxDA, PFODA, 6:2 diPAP, 8:2 diPAP, and PFOA.

This study demonstrates that PFAS are used to maintain water-repellency and grease-proof characteristics of various consumer products on both the Indonesian and U.S. markets. The data highlights the importance of urgent actions to prohibit the production, sale, and use of PFAS as a class and in all of its non-essential uses.

6. Acknowledgments:
Nexus3 and IPEN gratefully acknowledge the financial support provided by the government of Germany, the government of Sweden, and other donors that made the production of this document possible. The expressed views and interpretations herein shall not necessarily be taken to reflect the official opinion of any of the institutions providing financial support.
7. References:


Per- and Polyfluorinated Substances (PFAS): Occurrence and Exposure
S.Brandsma & Y.Fujii

MON-PM2-A4 PFAS in Selected Products in Indonesia

MON-PM2-B1 Identification of synthetic antioxidants and insights into their metabolism

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Introduction: Synthetic antioxidants (AOX) are a class of compounds sought as contaminants of emerging concern (CECs). The most frequently used classes of AOX are the low molecular weight synthetic phenolic antioxidants (LMW SPAs), the high molecular weight synthetic phenolic antioxidants (HMW SPAs), and the organophosphate antioxidants (OPAs). The HMW SPAs and OPAs are added to a high variety of consumer products, such as plastics, paints, and adhesives. Special attention should be paid to applications such as food contact materials (FCMs) and toys, as they pose a higher risk of potential human exposure to AOX. A recent study shows that AOX are present in car seats in the same concentration range as phosphate flame retardants (PFRs) and at significantly higher concentrations than brominated flame retardants [1] which underlines the need to gain more insights into the presence of AOX in consumer products. To this end, the implementation of suspect screening approaches can increase the range of AOX which can be identified and characterized.

In addition, metabolites of prevalent AOX should be identified to be able to accurately assess human exposure. This involves the identification of metabolites by in vivo models or alternatively by in vitro metabolism approaches such as incubation with human liver microsomes (HLM). To confirm a metabolite as a human biomarker, the compound has to be identified in a human matrix.

Materials and Methods: In the first step, FCMs (n=36) and toys (n=22) made from conventional plastics, novel plastics (e.g. polylactic acid), and synthetic rubber were screened for the presence of >80 AOX. The AOXs were extracted by ultrasonication and extracts were analyzed using liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS). Secondly, AOX standards were in vitro metabolized by incubation with HLM and human liver cytosol (phase I and II) in order to identify metabolites. Several positive and negative control samples were included. After incubation, samples were analyzed using HPLC-HRMS. In silico prediction data from Meteor Nexus v.3.1.0 were used to develop suspect lists containing possible metabolites. Lastly, phenozan was semi-quantified in 12 adults from Gdansk (Northern Poland) who provided monthly 24-hour urine samples (n=138) for one year. Samples were deconjugated and extracted by solid phase extraction prior to injection on liquid chromatography coupled to triple quadrupole mass spectrometry.

Results: Analysis of FCM and toys resulted in the identification of 5 HMW SPA, one OPA, and 3 degradation products with a confidence level of 1 (CL1) according to the identification scale of Schymanski et al. [2]. An additional 6 AOX were tentatively identified at CL2. Interestingly, more than 50% of the AOX were found in combination with other AOX. In 10% of the samples, 4 AOX were detected in the same material. Trends were also identified based on polymer, with the identification of AO1076 prevalent in FCM and toys were studied in vitro to identify their metabolites. This led to the identification of phenozan as a hydrolysis product of AO1076 and AO1024. In a similar hydrolysis reaction of AO245, methyl 3-(3-tert-butyl-4-hydroxy-5-methylphenyl)propanoate was identified. These hydrolyses did not require NADPH, but highly depended on HLM, strengthening the hypothesis that esterases catalyze the metabolism. Last, a quantitative method was developed for phenozan in human urine. Phenozan was detected in 99.28 % of urine samples, which confirmed that phenozan can be categorized as a human biomarker and not just a metabolite.

Discussion and Conclusion: The presented study showed for the first time that more than 3 AOX are used in one consumer product, indicating the wide variety of AOX used. The prioritized AOX were in vitro metabolized confirming that phenozan is a human metabolite as was hypothesized from Mabury et al. (2021) [3]. In addition, methyl 3-(3-tert-butyl-4-hydroxy-5-methylphenyl)propanoate was detected for the first time as a metabolite from AO245. These metabolization results led to the quantification of phenozan in human urine proving its application as a biomarker of multiple HMW antioxidants.

References:
15:40 - 17:00

Screening and Identification of Novel Contaminants
E. Schymanski & B. Le Bizec


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1. Introduction:
Tire-derived chemicals (TDCs) have come to the forefront of environmental science research due to evidence of acute toxicity to some species of fish (Tian et al., 2020; Brinkmann et al., 2022). Recent studies have revealed the presence of TDCs in ambient air and particulate matter/dust and concerns associated with inhalation exposure (Zhang et al., 2018; Zhang et al., 2022). Many TDCs are readily oxidized in air and transformation products have been reported and have added to concerns regarding potential cumulative effects (Yang et al., 2021, Cao et al., 2022). There is a need to better characterize the occurrence of TDCs and their products in urban air to evaluate their ecological and human health risks. Passive air samplers comprising polyurethane foam (PUF) disks (i.e., PUF-PAS) housed in stainless steel domes, have been applied successfully for tracking TDCs in urban air across different source sectors (Johannessen et al., 2023) and at the global scale (Johannessen et al., 2022). We summarize the findings from these studies and propose guidance for the application of PUF-PAS for investigating TDCs in air and suggest areas for future research.

2. Materials and Methods:
PUF-PAS samplers were deployed across 8 sites in the city of Toronto during 2016/2017, for 6 consecutive sampling periods of approximately 2-months duration. The PUF-PAS samplers are shown to be well suited for TDCs because they collect both gas-phase and particle-associated chemicals (Markovic et al., 2015). TDCs have been shown to span the spectrum of gas-particle partition in air – with some chemicals fully in the gas-phase while others entirely associated with particles (Johannessen et al., 2022b). PUF-PAS samplers that were deployed under the Global Atmospheric Passive Sampling (GAPS) Megacities project at 22 major cities were also screened for TDCs and provide a global perspective (Johannessen et al., 2022). All samples were analyzed by an UltiMate 3000 ultra-high pressure liquid chromatography system (UPLC) coupled to a Q-Exactive high resolution Orbitrap (Thermo Fisher, Waltham, MA, USA). Details on sample preparation and analysis are reported in Johannessen et al. (2022) and Johannessen et al. (2023).

TDCs targeted in this study include diphenyl guanidine (DPG), diphenylamine, hexa(methoxymethyl)melamine (HMMM), and 2,2,4-trimethyl-1,2-dihydroquinoline (TMQ) as well as select compounds from larger chemical classes that have been strongly associated with tire-wear, such as p-phenylenediamines (PPDs), benzothiazoles, and benzotriazoles.

3. Results:
Figure 1 summarizes dominant TDCs observed across eight sites in Toronto that are impacted by different source-sectors, during 2016-2017. High concentrations were observed for DPG, HMMM, and various benzothiazoles and benzotriazoles. 6PPD was at low concentrations or below detection at most sites while 6PPD-Q was only detected at the two traffic sites. These results are generally consistent with results from more than 20 megacities sampled in 2018 (Johannessen et al., 2022), highlighting the global-scale nature of this class of chemicals.

Many of the TDCs, as indicated by the blue ovals in Figure 1, were detected and/or elevated at the two sites that have substantial traffic influence. Site MOE is within 10s of meters of Canada’s busiest stretch of highway (Highway 401) and site WB is located in a commercial intersection in downtown Toronto. Principal components analysis was applied to the data (Figure 2) and show that the two traffic-impacted sites are distinct from the other 6 source sector sites and support traffic as a key source of emissions for these chemicals.

The TDCs also showed a distinct pattern of elevated concentration in the winter sampling periods (P3 and P4) compared to the other seasons. This suggests that there are important differences in seasonal emissions of TDCs with potential impacts on urban air quality during colder months.
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Figure 1: Concentration in air heatmap of tire-derived chemicals from PUF-PAS deployed in Toronto source-sector sites in consecutive sampling periods starting in Oct. 2016 (period 2, P2) and ending in September 2017 (P6); FB = field blank. Blackened square represents non-detects (<IDLs) or detects <MDLs. BUR, DV etc are site names and icons at the top of the figure indicated predominant source types for each site.

Figure 2: Principal component (PC) analysis of tire-derived chemicals in source-sector resolved and seasonal dataset. PC1 and 2 account for 45% and 11% of the variability in the dataset, respectively.
4. Discussion:
Concentrations in air for TDCs, derived from PUF-PAS samplers should be considered semi-quantitative due to uncertainties, which include:

i.) variability in PUF-PAS sampling rate, which is on the order of $4 \text{ m}^3/\text{day} \pm 2 \text{ m}^3/\text{day}$. The default $4 \text{ m}^3/\text{day}$ was applied in this study;

ii.) uncertainties regarding the stability of TDC on PUF-PAS during the 2-month deployment periods;

iii.) uncertainties in the uptake profile of TDCs, particularly those with higher volatilities (lower log $K_{OA}$ values) which may be prone to equilibration in the PUF-PAS sampler during a 2-month deployment, resulting in lower effective air sample volumes compared to less volatile (higher $K_{OA}$) TDCs, which do not approach equilibrium in the PUF disk and remain in the linear uptake region for the entire 2-months.

Figure 3 (left panel) plots the $K_{OA}$-model (Harner and Bidleman, 1998) derived gas-particle partition coefficient for selected TDCs based on their $K_{OA}$ values at 25 °C. The right panel shows PUF-PAS uptake profiles as a function of log $K_{OA}$. These results indicate that the assumption of linear-phase sampling for PUF-PAS may not be appropriate for all TDCs (e.g., benzotriazole and benzothiazole) and may overestimate effective air sample volumes, thereby under-estimating PUF-PAS derived concentrations in air.

![Figure 3: Gas-particle partitioning of selected TDCs using the $K_{OA}$ model (Harner and Bidleman, 1998) using TSP=10 mg/m$^3$ and $f_{omp}=0.02$; right panels showing uptake profile for PUF-PAS samplers as a function of log $K_{OA}$. Log $K_{OA}$ values taken from Johannessen et al., 2022b, Cao et al., 2022 for 6PPD and 6PPD-Q, and Johannesen and Parnis, 2021 for HMMM.](image)

5. Conclusions:
Surveys of urban air using PUF-PAS samplers have revealed the ubiquitous presence of TDCs in the vicinity of different sources in Toronto and that traffic-related emissions are likely the major source to air. PUF-PAS samplers are well suited to capture TDCs in air which span a wide range volatilities and gas-particle partitioning. More work is required to confirm the performance of PUF-PAS samplers for TDCs and their transformation products, including calibration against active samplers and assessing PUF-air partition coefficients so that accurate uptake profiles and concentrations in air can be derived. Studies on the stability of TDCs collected on PUF-PAS are also needed.

6. Acknowledgments:
Partial funding for this work was provided by the Chemicals Management Plan.

7. References:
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MON-PM2-B3 Assessment of the toxic potency and mutagenicity of soils from waste dumping sites in Wallonia, Belgium

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1. Introduction:
Soil pollution caused by human activities is an alarming issue that puts pressure on soil health and its capacity to provide ecosystem services. Thousand man-made chemical compounds with potential toxicity have been released and deposited on the earth's surface, posing a great threat to human, plant, and animal health. Some pollutants break down in soils over time, however others such as persistent organic pollutants [Polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs), biphenyls (DL-PCBs) and polycyclic aromatic hydrocarbons (PAHs)] remain for very long times. The latter, even at low exposure levels, can exert toxic responses such as immunotoxicity, developmental and reproductive toxicity, neurotoxicity, and carcinogenicity. Moreover, they can be endocrine disrupting chemicals altering the processes and functions of the endocrine system (Lohmann et al., 2007). Most of the toxic actions of these compounds are mediated via AhR signal transduction pathway (Safe, 1990 typified by the polychlorinated dibenzo-p-dioxins (PCDDs); Koppen et al., 2001). They can bind to the AhR receptor and alter biochemical and cellular functions at cellular level. The full risks of these chemicals and their various mixes are not yet fully known. In this study, the toxic potency and the mutagenicity of high risk contaminated soils from waste dumping sites in Wallonia, Belgium were assessed. A combined approach of bioanalytical assays (AhR/ER – CALUX) and the Ames test was applied in the subset of soil samples collected in 2020-2021 from seven sampling points in Wallonia, Belgium.

2. Materials and Methods:
Six soil samples (denoted as S1-6) were collected from six different heavily industrialized dumping sites in Wallonia region in Belgium. One reference sample (Sample 7) was collected from a non-contaminated area, in the countryside of south Wallonia. The Chemically Activated LUciferase gene eXpression (CALUX) method is used to assess on cellular level the toxic potency of (mixtures of) chemical compounds, such as PCDD/Fs, dl-PCBs, and PAHs (Baston & Denison, 2011) 3,7,8-tetrachlorodibenzo-p-dioxin and related dioxin-like halogenated aromatic hydrocarbons in a wide variety of sample matrices. While sample extracts containing complex mixtures of chemicals can produce a variety of distinct concentration-dependent luciferase induction responses in CALUX cells, these effects are produced through a common mechanism of action (i.e. the Ah receptor (AhR). In this case study, mouse hepatoma cells (H1L7.5c1) and human breast carcinoma cells (VM7Luc4E2) with cellular receptors, aryl hydrocarbon receptor (AhR) and estrogenic receptor (ER) respectively, were used to investigate their binding affinity to the chemicals present on the soil samples. The bioassay provides a biological response to all receptor active compounds in the sample including the unknown compounds and their cocktail effect (antagonistic and/or synergistic effects of the various compounds between them). Results are expressed "biological equivalence concentrations" (BEQ) that indicates the biological response as a concentration of a reference compound [Benzopyrene (BaP), 2,3,7,8-tetrachlorodibenzo-dioxin (TCDD) and 17 estradiol (E2)], resulting in the same response (Villeneuve et al., 2000)there is a need to calculate and present REPs in a manner that addresses the potential uncertainties caused by violation of the assumptions of parallelism and equal efficacy. Multiple point estimates, over the range of responses from EC20 to EC80, can be used to derive relative potency ranges (REP20–80 range; Escher et al., 2015) we propose a statistical method to read directly across from chemical guideline values to trigger values without the need to perform in vitro to in vivo extrapolations. The derivation is based on matching effect concentrations with existing chemical guideline values and filtering out appropriate chemicals that are responsive in the given bioassays at concentrations in the range of the guideline values. To account for the mixture effects of many chemicals acting together in a complex water sample, we propose bioanalytical equivalents that integrate the effects of groups of chemicals with the same mode of action that act in a concentration-additive manner. Statistical distribution methods are proposed to derive a specific effect-based trigger bioanalytical equivalent concentration (ETB-BEQ). The CALUX method has already been widely used to screen dioxin- or estrogen-like compounds in different matrices such as water, sediments, dust, foodstuffs, animal feed, etc (Vandermarken et al., 2018) crossing the Brussels region (Belgium; Vandermarken et al., 2016) it is difficult to assess and manage possible human health risks. For young children, who are particularly vulnerable to endocrine disruption due to their development rate, indoor dust is one of the main routes of exposure. In this study, an estrogen responsive elements chemically activated luciferase gene expression (ERE-CALUX; My et al., 2021; Scippo et al., 2004) it was foreseen in the EU strategy to integrate screening methods, using either a qualitative (screening).
A parallel evaluation of the potential genotoxic/mutagenic contaminants in soil samples was performed with the Ames test (the bacterial reverse gene mutation test) using Salmonella typhimurium. This method is able to detect a wide range of chemical compounds that can cause damage and lead to gene mutations (Mortelmans & Zeiger, 2000). In this study, the Ames tests were performed in typhimurium TA100 (detection of base pair substitutions) and Salmonella typhimurium TA98 (detection of frameshifts) with and without an external metabolic activation system. Using this combination of these two bacterial strains, can detect up to 90% of mutagens.

Targeted compounds were extracted from the freeze-dried and homogenized soil samples in order to analyze them with both CALUX bioassay and the Ames test. A manual extraction method, involving ultrasonication and Vortexing (Baston & Denison, 2011) 3,7,8-tetrachlorodibenzo-p-dioxin and related dioxin-like halogenated aromatic hydrocarbons in a wide variety of sample matrices. While sample extracts containing complex mixtures of chemicals can produce a variety of distinct concentration-dependent luciferase induction responses in CALUX cells, these effects are produced through a common mechanism of action (i.e. the Ah receptor (AhR was used as using ASE (Accelerated Solvent Extractor) was not possible due to the high content of contamination on the soils that resulted in several times blockage of ASE injectors. The materials used for the extraction consist of celite columns (25ml), glass whole, sodium sulphate (Na₂SO₄), n-hexane, methanol, and toluene. PCB and Dioxin fractions were obtained from the soil sample extracts from a clean-up procedure using a combination of two columns. The acid silica column (10ml) consists of a plug glass of wool, 0.5cc sodium sulphate, 1.6cc silver nitrate 10% on silica gel, 4.3cc of 33% (w/w) sulfuric silica gel and 0.5cc sodium sulphate. On the other hand, the carbon column (10ml) is packed with a plug of glass wool, 0.5cc of sodium sulphate, 1cc X-CARB (packed) and 0.5cc sodium sulphate. Other materials used for the clean-up are n-hexane, acetone, toluene, ethyl acetate and sulphuric acid.

3. Results:

![Figure 1: AhR-CALUX results for crude soil extract expressed in µg BEQ-BaP g⁻¹ dw](image1)

![Figure 2: AhR-CALUX results for cleaned-up soil extract for PCDD/Fs and dl-PCBs expressed in pg BEQ-TCDD g⁻¹ dw](image2)
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Table 1: Ames test results for TA100, without and with S9 (metabolic fraction)

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<th>Sample</th>
<th>Without S9</th>
<th>TA100</th>
<th>With S9</th>
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<td>Concentration (mg/plate)</td>
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<td>6.25</td>
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<tr>
<td>Concentration (mg/plate)</td>
<td>SC</td>
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<td>10%</td>
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Table 2: Ames test results for TA98, without and with S9 (metabolic fraction)

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<th>Sample</th>
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<th>With S9</th>
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<tr>
<td>Concentration (mg/plate)</td>
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<td>6.25</td>
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<td>Sample 2</td>
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</tr>
<tr>
<td>Sample 7</td>
<td>13</td>
<td>11</td>
<td>23</td>
</tr>
</tbody>
</table>

Note: PC (Positive Control): *4NQO 0.2yg/plate - 2-aminoanthracene 1yg/plate
Values in red = # revertants >= 2x # revertants SC (Solvent Control)

4. Discussion:
In this study, the CALUX bioassay was conducted using both crude (total) soil sample extract (containing all extracted compounds) to determine the total AhR activity for a sample and cleaned-up soil extracts (samples with PCDD/Fs and dl-PCBs isolated from the total extracts) to determine the contribution of the PCDD/Fs and dl-PCBs to the total AhR activity. The results are presented in Figure 1 and Figure 2 respectively, expressed in "biological equivalence concentrations" (BEQ) of a reference compound. The AhR ligand activation on CALUX for the investigated soil samples from heavily industrialized dumping sites in Wallonia region in Belgium, is found significantly high in comparison with the reference non-contaminated sample (Sample 7). For the AhR-CALUX all samples were positive, indicating the presence of active AhR agonists in soil extracts. The AhR toxicity potency estimates ranged from <3 to 140±28 µg BEQ-BaP g−1 dw for crude extract (Figure 1), <4 to 613±88 pg BEQ-TCDD g−1 dw for PCDD/Fs (Figure 2), <18 to 241±35 pg BEQ-TCDD g−1 dw (Figure 2) for dl-PCBs. As expected, the AhR activity for the crude extract shows higher values. Overall, sample S3 shows the highest biological activity with respect to the effect of the present chemicals on the soil sample. Then the samples S4, S5, and S2 follow with approximately similar biological response levels. Last, samples S1 and S6 show lower values of AhR toxic potency response compared to the other samples and the reference sample S7 shows the lowest values as expected. The differences that we see among the samples, can be attributed to the sources of pollution of the studied soils (type of waste dumped in sample collection area), longevity of waste, area/location of the dumping site, season of sample collection and potential other factors.

The ER-CALUX potency estimates were below determination levels. However, antagonistic activity (blocker) was detected, which could prevent full determination of the agonist activity/potency.

The mutagenicity tests (Ames tests) of the soil samples conducted in Salmonella typhimurium strains TA100, in the presence and absence of the S9 metabolic activation revealed only Sample S2 mutagenic (Table 1). On the other hand, the mutagenicity tests achieved in Salmonella typhimurium strains TA98, in both the presence and the absence of the S9 metabolic activation, show most of the soil samples (S2-6) mutagenic, except sample S1. The results of both methods are well correlated with each
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other and indicate mutagenicity and toxic potency of these soil samples due to the chemical contamination. In Belgium, a study done in estuarine sediments via CALUX bioassay shows values of <1-60 pg BEQ-TCDD g⁻¹ dw for PCDD/Fs (Vandermarken et al., 2018). Crossing the Brussels region (Belgium). In other countries, soils investigated via CALUX, show PCDD/Fs values such as 2-44 pg BEQ-TCDD g⁻¹ dw in China from incineration plants waste (Du et al., 2011), 1-650 pg BEQ-TCDD g⁻¹ dw in Korea (Joung et al., 2007) Chemically Activated Luciferase gene eXpression bioassay, has proven valuable for screening for and assessing toxic equivalents of dioxin-like compounds, because it detects all AhR (arylhydrocarbon receptor), <30-4000 pg BEQ-TCDD g⁻¹ dw in Vietnam from e-waste processing area (Suzuki et al., 2016) which can be a source of both useful materials and toxic substances, depending on the processing method, is important for promoting material recycling. In this study, we used the dioxin-responsive chemical-activated luciferase gene expression (DR-CALUX, and <3-2727 pg BEQ-TCDD g⁻¹ dw in Vietnam, from a A-So airbase ("restricted zone") with no agriculture activity due to the heavy dioxin contamination (My et al., 2021). By comparing the data of our study with these other studies (above mentioned), we can say that the dioxin levels are alarming, and that further risk assessment should be considered. A combination of the Ames test and in vitro mammalian cell genotoxicity assays was able to detect almost all of the 962 rodent carcinogens and in vivo genotoxins tested in a study by (Kirkland et al., 2014). In our case study, almost all the soil samples from dumping sites in Wallonia (S1-6) were found positive for both AhR-CALUX and the Ames tests, which can be correlated to a carcinogenic potential. Thus, the investigated in this study Wallonia waste dumping sites, should be considered as dioxin hotspots with carcinogenic potential and should be subject of systematic monitoring for these chemicals.

5. Conclusions: The soils investigated in this study show critical levels of toxic and mutagenic activity. Routine assessments and monitoring of un/known chemicals with potential adverse effects on the environment, wildlife, and human health should be of crucial importance. These polluted soils can be an input pathway of organic contaminants to the ecosystems, harming human health, wildlife, and negatively affecting food, water, and air quality. Currently the toxicity of chemical substances in soils is mainly evaluated using a substance-by-substance approach. However, despite the detailed quantification of several congeners that chemical instrumental analysis offer, there is also shortcomings such as not being able to analyze all substances present in environmental matrices, and their potential mixture effects, making it very challenging to use the data for future predictions. Effect-based method (EBM) can be used to overcome the challenges identified above. To ensure protection of soil quality we should be able to understand the potential for effects caused by the sum of the chemical substances present in them and translate the observed effects in cost-effective management options. The in vitro genotoxicity testing, such as what was achieved in this study, as a combination of the in vitro CALUX bioassay and the Ames test provides a suitable and additional support in chemical risk assessment. Based on the findings of this study, future systematic monitoring of hazardous chemicals in soils is strongly recommended.

6. Acknowledgments: We would like to express our gratitude to AWEC (Agence wallonne de l’air et du climat) for funding this project and providing the soil samples to VUB. We would also like to thank Scienano for the Ames tests and their appreciated support and advice.

7. References:
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Bharat Chandramouli, Million Woudneh, SGS

Introduction:
N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) is added to almost every automobile tire to prevent cracking from ozone. However, a newly discovered environmental reaction by-product 6-PPD-quinone (6-PPDQ) has been recently identified as being responsible for urban runoff mortality syndrome (URMS) in Coho Salmon (Oncorhynchus kisutch). The LC-50 of 6-PPDQ is 41 ng/L to juvenile Coho making it one of the most toxic substances known to us. In addition to extreme toxicity in Coho, significant lethal effects (LC-50 1 µg/L or less) has been observed in geographically widespread species such as brook trout (Salvelinus fontinalis) and rainbow trout (Oncorhynchus mykiss). The toxicity shows 5 orders of magnitude species dependence. Some of the first 6PPD-quinone environmental monitoring results have started to appear in the open literature.

In this study, we present results from method development and validation of a very sensitive UPLC-MS/MS method, stability studies and environmental levels of 6PPD and 6PPD-quinone in surface water runoff.

Materials and Methods:
We developed an isotope-dilution UPLC-MS/MS method in aqueous samples. 6PPDq was extracted from whole water using dichloromethane (DCM). The resulting extract was cleaned up using a silica SPE cartridge. The extract was solvent exchanged to acetonitrile and analyzed by UPLC-MS/MS. The method was validated for accuracy and precision in reagent water (10 ng/L spike n =5) and for robustness of performance in surface and groundwater. To determine the storage requirements of 6PPDq, suitable containers and sample hold times were investigated. For studying stability, reagent water and river water samples were spiked at 70 ng/L and stored for a period of up to 35 days (at analysis intervals of 0, 3, 7, 14 and 35 days). Extract stability studies were performed at 28-day time point after storage at –20, +20 and 4°C. We then applied the method to multiple studies in coastal British Columbia Canada and other locations.

Results:
The method validation resulted in a reporting limit of 0.1 ng/L (400 times lower than LC-50) for 6PPDq and semi-quantitative analysis of 6-PPD, which was found to be very unstable. The method showed a mean recovery of 100% and RSD values of 0.6% for 6PPDQ. Robustness testing indicated no significant matrix effects. Container studies showed that the HDPE container is not suitable for the collection and storage of 6PPDq in water due to strong adsorption to the container walls. Reagent water and river water samples stored for a period of up to 35 days demonstrated no significant change in the concentration of the analyte. Extract stability study demonstrated that 1:1 acetonitrile:methanol extracts were stable for 28 days. The occurrence results from coastal British Columbia, Canada and adjacent regions showed that 94% and 88% of the aqueous samples showed detected levels of 6PPD-quinone and 6PPD respectively. The measured concentrations ranged from 0-740 ng/L for 6PPD-quinone and 0-5100 ng/L for 6PPD. On average, the concentration of 6PPD was 3.5x that of the 6PPD. The highest concentrations were detected in the wet season, and the lowest concentrations were detected in the dry season.

Discussion and Conclusions:
Our method validation and initial occurrence data shows the widespread occurrence of 6PPDQ at levels toxic to multiple species and points to increased need for the monitoring of 6PPDQ, and the measurement of toxicity in other species given the wide variance in toxicity by species.
1. Introduction:
Persistent Organic Pollutants (POPs) are substances of international concern because they are resistant to environmental degradation through chemical, biological, and photolytic processes. Due to their persistence, POPs bioaccumulate with potential adverse impacts on human health and the environment. For these reasons, the Stockholm Convention (SC) on POPs, which is a global treaty, has a pursuit to protect human health and the environment (UNEP, 2014). Despite their usage in the last decade, questions about their role as vectors enhancing the bioaccumulation of POPs in aquatic environments are still pending (Rochman, 2015; Lusher, 2015). POPs are associated with numerous health issues in humans, including endocrine disruption, cancer, obesity, cardiovascular disease, and reproductive issues, to name a few (Alharbi et al., 2018). In nature, complex mixtures of toxicants are present and can be transferred, causing synergic interactions between them to enhance toxicity in the organism (Jung et al., 2018). The objective of our study was to investigate the levels and spatial distribution of Persistent organic Pollutants (POPs) such as Dichlorodiphenyltrichloroethane (DDTs), Hexachlorocyclohexane (HCHs) and Polychlorinated biphenyl (PCBs) contamination in the water along BioBío River. This was aimed at obtaining information on the potential sources of contamination and detail data on the transport of contaminants during the different seasons of the year.

2. Materials and Methods:
The Biobío River basin is located in central Chile with a surface area of 24,260 km² and located between 36°45' and 39° south latitude. Its flow rates range between 120 and 8,800 m³ s⁻¹ (Habit et al., 2006). The sampling campaign was conducted in central Chile along the Biobío River (36°26'S; 38°29'S - 72°10'W; 73°40'W) and Itata river (36°23'S - 72°51'O) in 2 different campaigns (January 2022, and July 2022). The total of sampling sites was divided as rivers of the Upper zone (BB1: Santa Barbara River, BB2: Duqueco River, BB3: Cholhuague River, BB4: Bureo River), rivers of the Middle zone (BB5: Vergara River, BB6: Huaque River, BB7: Laja River, BB8: Claro River) and rivers of the Lower zone (BB9: Site before Mochita (potable water treatment plant), BB11: Site after wastewater treatment plant (potable water treatment plant), BB12: BioBío mouth, IT: Itata mouth, IT2: Itata River (inside).

3. Results:
DDT is an organochlorine pesticide (OCP) and colorless. It is very soluble in fats and in organic solvents, and practically insoluble in water and with a high persistence for a long time in the environment and accumulates in the food chain and in the tissues of organisms (Jacob, 2013). The results obtained showed that in the summer (Fig. 1), DDTs concentrations were from 0.003 to 0.019 (0.006 ± 0.001) ng/L. The maximum was in BB6 (Upper zone). HCHs are organochlorine insecticide. The most extensive use of technical HCH was during the 1970s and early 1980s followed by a rapid decrease as a result of restrictions and prohibitions in many countries (Li et al., 2000; Pozo et al., 2020). DDTs and HCHs have been causing widespread concern, despite effective control on their manufacturing, agricultural and vector practices (Saadati et al., 2012). The range value was from 0.006 to 0.042 ng/L. The maximum value found for the sum of PCBs was in the IT station (0.040 ng/L) (0.009 ± 0.011 ng/L), and the minimum was 0.002 ng/L (0.004±0.005 ng/L) in the IT2 station (Lower zone).

Fig. 1: Levels (ng/L) of POPs (DDTs, HCHs, PCBs) in Upper, Middle, and Lower zone in Summer
For the winter campaign (Fig. 2), the maximum value found for the sum of the DDTs was from 0.003 to 0.535 ng/L (0.171 ± 0.061). The maximum was in station BB6 (Middle zone). The maximum value found for the sum of HCHs was from 0.004 to 0.026 ng/L (0.017 ± 0.012 ng/L). The maximum was in BB7 (Middle zone). And the maximum value found for the sum of PCBs was from 0.003 to 0.097 ng/L (0.016 ± 0.022 ng/L). The maximum was in BB7 (Middle zone).

4. Discussion:
Our DDTs results are lower than what was found in Gujarat State, India (4.27 ng/l and 7.56 ng/l) by Kashyap et al., 2001, in Ebro River (Spain) (2–6.8 ng/L), by Fernandez et al., 1999, and in the Qiantang River in East China (0.4–97.54 ng/L) reported by Zhou et al., 2006. In the case of HCHs, levels in the BioBío River are also lower than those data reported in lower compared with, where the HCHs levels were in new ice sampled in the Canadian High Arctic (0.642 ± 0.046 ng L–1) found by Pućko et al., 2009, in Gujarat State, India (5.53 ng/l and 6.96 ng/l) by Kashyap et al., 2001, in the Daliao River, China (3.4-23.8 ng/L) by Tan et al., 2009. The PCBs concentrations in this study are lower than PCBs found in the southeast coast of China (0.2–985.2 ng/L) by Han & Currell, 2017, then levels found in the sub-surface water samples collected at two sites of the Venice Lagoon (Italy) (0.45 ng/l to 2.1 ng/l) by Manodori et al., 2006.

5. Conclusion
The present study provides new information of occurrence, spatial-temporal distribution, of POPs along Biobío region, in central Chile. From the two River’s mouth analyzed IT (Itata mouth; Lower zone) registered the higher levels of compound sampled compared to the Middle and Upper zone, this could be caused by the industrial and urban areas. Further research is ongoing to assess the likely impact of chemicals substances in the coastal areas.

6. Acknowledgements
This study was funded by Agencia Nacional de Investigación y Desarrollo (ANID) through projects Fondecyt 1161673 (KP), 1211931 (KP). The authors thank the RECETOX RI (No LM2023069) financed by the Ministry of Education, Youth and Sports of the Czech Republic, and Operational Programme Research, Development, and Education – project CETOCOEN EXCELLENCE (No CZ.02.1.01/0.0/0.0/17_043/0009632) for supportive background.

7. References:


1. Introduction:
Medical waste is all the waste generated by healthcare facilities, medical laboratories and biomedical facilities, as well as waste from home healthcare. The bulk of healthcare waste is produced by hospitals. It is universally accepted as a potential danger to human health and environment if it is not managed in an environmentally safe manner. Improper treatment and disposal of medical (healthcare) waste pose serious risks of disease transmission due to exposures to infectious agents among waste pickers, waste workers, health workers, patients, and the community in general. Not all wastes generated from healthcare facilities are infectious and/or hazardous. Between 75% and 90% of the waste produced by healthcare facilities is comparable to domestic waste and usually called "non-hazardous-waste". Open burning and incineration without adequate pollution control exposes waste workers and the community to toxic contaminants in air emissions and bottom ash. These contaminants include unintentionally produced persistent organic pollutants (UPOPs) such as polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs), dioxin-like polychlorinated biphenyls (dl PCBs), hexachlorobenzene (HCB), pentachlorobenzene (PeCB) and recently also some per- and polyfluoroalkyl substances (PFASs). Waste incineration is listed among major sources of UPOPs such as PCDD/Fs in Annex C to the Stockholm Convention. PCDD/Fs, dl PCBs and chlorinated benzenes have been observed in emissions to air as well as in bottom ash, fly ash from medical waste incinerators (MedWIs). Emission factors for PCDD/Fs were established in the Dioxin Toolkit based on previous studies. High levels of PCDD/Fs were also observed in residues from simple, batch-type MedWIs. There has also been observed contamination of the food chain in the vicinity of small MedWIs not limited to developing countries. However waste incinerators burning healthcare waste in developed countries may also burn other wastes. Therefore local contamination cannot be linked only to incineration of medical waste in such cases.

In this study, we focused on five recently sampled sites of MedWIs in Africa and present findings on changes in congener profile and concentration of UPOPs in bottom ash and other residues. We also present evidence of PCDD/Fs and dl PCBs in food chains by monitoring free range chicken eggs from within the vicinity of MedWIs and compared the levels with those sampled in 2018-2019 and 2004-2005. We also discuss the implications and meaning of our findings in relation to implementation documents of international environmental conventions.

2. Materials and Methods:
We have collected two samples of bottom ash and one sample of soot from small MedWIs located in Ghana and Gabon in 2018 and 2019. These were always pooled samples of bottom ash or soot from at least 5-point samples which were homogenized. Samples were taken either from the bottom ash pile next to waste incinerator or from incineration chimney (soot) by stainless steel shovel and homogenized in a stainless-steel bowl. Samples were kept in polyethylene sample boxes and transported under cold conditions to laboratories in State Veterinary Institute and University of Chemistry and Technology in Prague. We also included results of the analyses for POPs in free-range chicken eggs in this study for comparison of the potential food chain contamination in the surroundings of small MedWIs up to 3000 tons of waste/year capacity. These results were already presented in our previous studies but were not studied with a focus on MedWIs.

All samples were analyzed for their content of individual PCDD/Fs and dl PCBs by GC/HRMS in an ISO 17025 accredited laboratory with a resolution >10,000 using 13C isotope labelled standards. PCDD/F and dl-PCB analysis in eggs followed the methods of analysis for the control of levels of PCDD/Fs and dl-PCBs in foodstuffs according to EU regulations. The results are presented in pg WHO TEQ/g (per g dry matter for ash and per g fat for eggs). TEFs defined in 2005 were used to evaluate dioxin toxicity in all samples.

Analyses of HCB were conducted in a Czech certified laboratory at the University of Chemistry and Technology in Prague. The analytes were extracted by a mixture of organic solvents, hexane: dichloromethane (1:1). The extracts were cleaned by means of gel permeation chromatography (GPC). The identification and quantification of the analyte was conducted by gas chromatography coupled with tandem mass spectrometry detection in electron ionisation mode for HCB. Sampling and analyses of the eggs were described in more details in previous studies.
The carry-over rates which were determined by the Dutch National Laboratory\textsuperscript{19} were used for re-calculation of PCDD/F and PCB congener profiles in eggs for better comparison with profiles in ash and soil samples. The carry-over rates for PCDD/Fs range from 44\% (TCDD) to 10\% (OCDD). The transfer factors for dl-PCBs are, on average, high and ranged from 80\% (PCB-167) to 41\% (PCB-81). For some comparison with source patterns, these carry-over rates are used to roughly recalculate the measured PCDD/F and PCB data in eggs\textsuperscript{20}.

Analytical results of bottom ash analyses from a previous study in Pakistan\textsuperscript{21} focused on sampling at batch-type MedWIs and from the study focused on small MedWI and open pit burning of medical waste in Mozambique\textsuperscript{22} were also included for comparison. Sampling and analytical methods for bottom ash samples from Pakistan and Mozambique are described in previous studies.\textsuperscript{21,22} They were similar to those presented in this study.

Sampling sites in Cameroon, Ghana, and Gabon were small batch-type MedWIs mostly operated several times per week. There was also an open pit for burning medical waste next to small waste incinerator in Yaoundé, Cameroon. MedWI located in Accra, Ghana stopped operation several years before sampling, but a bottom ash pile was left next to the obsolete building and was sampled in 2018. Free-range eggs were sampled at Yaoundé hospital location in Cameroon. MedWI in Mozambique was described as having two burn chambers; a primary chamber where temperatures can reach up to 500 °C and a secondary chamber where temperatures can reach up to 800 °C. Propane gas is used for start-up and then the entire combustion process runs on diesel fuel.\textsuperscript{22} All above-described waste incinerators do not have any air pollution control systems. See also photos of some of sampled sites at Figures 1 – 6 below.
3. Results:
Measured levels of PCDD/Fs and dl PCBs in bottom ash samples and one soot sample from small MedWIs included in this study are summarized in Tables 1 and 2. HCB and PeCB were analyzed in bottom ash samples from African waste incinerators in addition to PCDD/Fs and dl PCBs. HCB and PeCB were measured at levels of 4.1 and 3.5 and 46 and 83 ng/g dw in samples from Accra – hospital and Nkoltang respectively.

Table 1: Comparison of PCDD/F and dl PCB levels in MedWI residues from African countries. Source of the data for Mozambique MedWI.

<table>
<thead>
<tr>
<th>Country</th>
<th>Ghana Locality</th>
<th>Gabon Locality</th>
<th>Mozambique Locality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Localities</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Accra - hospital</td>
<td>Kumasi - hospital</td>
<td>Nkoltang MedWI</td>
</tr>
<tr>
<td>Matrix</td>
<td>ash</td>
<td>soot</td>
<td>ash</td>
</tr>
<tr>
<td>PCDD/Fs (pg TEQ/g dw)</td>
<td>551</td>
<td>2315</td>
<td>2151</td>
</tr>
<tr>
<td>DL PCBs (pg TEQ/g dw)</td>
<td>28</td>
<td>100</td>
<td>106</td>
</tr>
<tr>
<td>PCDD/F + DL PCBs (pg TEQ/g dw)</td>
<td>579</td>
<td>2414</td>
<td>2257</td>
</tr>
</tbody>
</table>

Table 2: PCDD/F and dl PCB levels in bottom ash samples from Pakistani MedWIs.

<table>
<thead>
<tr>
<th>Country</th>
<th>Pakistan Locality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Localities</td>
<td></td>
</tr>
<tr>
<td>Matrix</td>
<td>ash</td>
</tr>
<tr>
<td>PCDD/Fs (pg TEQ/g dw)</td>
<td>2105</td>
</tr>
<tr>
<td>DL PCBs (pg TEQ/g dw)</td>
<td>155</td>
</tr>
<tr>
<td>PCDD/F + DL PCBs (pg TEQ/g dw)</td>
<td>2260</td>
</tr>
</tbody>
</table>

Figures 7 – 8: PCDD/F and dl PCB congener patterns in samples of free-range eggs and related bottom ash from two African sites, Accra – hospital (Ghana) and Nkoltang – MedWI (Gabon).
Table 3: POPs in free-range chicken eggs sampled in the vicinity of African MedWIs in comparison with reference egg sample from Accra supermarket. Samples from the years 2018-2019.

<table>
<thead>
<tr>
<th>Country</th>
<th>Cameroon</th>
<th>Ghana</th>
<th>Ghana</th>
<th>Gabon</th>
<th>Ghana</th>
</tr>
</thead>
<tbody>
<tr>
<td>Locality</td>
<td>Yaoundé-hospital</td>
<td>Accra - hospital</td>
<td>Kumasi - hospital</td>
<td>Nkoltang MedWI</td>
<td>Accra-supermarket</td>
</tr>
<tr>
<td>Eggs in pooled sample</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Fat content (%)</td>
<td>14.6</td>
<td>12.3</td>
<td>14.7</td>
<td>13.6</td>
<td>8.8</td>
</tr>
<tr>
<td>PCDD/Fs (pg TEQ/g fat)</td>
<td>4.6</td>
<td>49</td>
<td>1.74</td>
<td>11.5</td>
<td>0.39</td>
</tr>
<tr>
<td>DL PCBs (pg TEQ/g fat)</td>
<td>6.8</td>
<td>14</td>
<td>0.86</td>
<td>3.2</td>
<td>0.17</td>
</tr>
<tr>
<td>PCDD/F + dl PCBs (pg TEQ/g fat)</td>
<td>11.4</td>
<td>63</td>
<td>2.60</td>
<td>14.7</td>
<td>0.56</td>
</tr>
<tr>
<td>HCB</td>
<td>1.43</td>
<td>3.63</td>
<td>0.76</td>
<td>0.93</td>
<td>&lt;0.1</td>
</tr>
</tbody>
</table>

Table 4: POPs in free-range eggs from the vicinity of small MedWIs – samples from the years 2004–2005.

<table>
<thead>
<tr>
<th>Country</th>
<th>India</th>
<th>Philippines</th>
<th>Czech Republic</th>
<th>Czech Republic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Locality</td>
<td>Lucknow - hospital</td>
<td>Aguado MedWI</td>
<td>Beneshov – hospital</td>
<td>Lysa nad Labem MedWI</td>
</tr>
<tr>
<td>Number of eggs in pooled sample</td>
<td>4</td>
<td>6</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Fat content (%)</td>
<td>12.5</td>
<td>12.5</td>
<td>8.2</td>
<td>9.4</td>
</tr>
<tr>
<td>PCDD/Fs (pg TEQ/g fat)</td>
<td>18.5</td>
<td>8.1</td>
<td>3.0</td>
<td>3.8</td>
</tr>
<tr>
<td>DL PCBs (pg TEQ/g fat)</td>
<td>6.4</td>
<td>0.28</td>
<td>2.7</td>
<td>18.4</td>
</tr>
<tr>
<td>PCDD/F + DL PCBs (pg TEQ/g fat)</td>
<td>25</td>
<td>8.4</td>
<td>5.7</td>
<td>22.2</td>
</tr>
<tr>
<td>HCB</td>
<td>3.8</td>
<td>1.70</td>
<td>15</td>
<td>46</td>
</tr>
</tbody>
</table>

Levels of PCDD/Fs, dl PCBs, and HCB in free-range eggs from the vicinity of small MedWIs from developing countries are summarized in Table 3. They are compared with a reference sample of eggs from a supermarket in Accra and with free-range chicken eggs from the vicinity of MedWIs collected in 2004-2005 (see Table 4). PCDD/F and dl PCB congener patterns in samples of free-range chicken eggs and bottom ash from Accra – hospital and Nkoltang are presented in graphs at Fig. 7 and 8. Recalculation used for eggs is explained in Materials and Methods.

4. Discussion:
Levels of PCDD/Fs are by an order of magnitude higher than dl PCBs in bottom ash and soot samples from small MedWIs from developing countries presented in this study. Levels of PCDD/Fs in bottom ash samples from MedWI range from 38 to 2151 pg WHO-TEQ/g dw in this study and are from Class 1 and 2 MedWIs according to Dioxin Toolkit classification\(^{10}\). We compared them with other data for MedWI bottom ash samples available in literature in Table 5. Measured levels of PCDD/Fs are comparable to those from the waste incinerators of the same class in Thailand\(^{23}\) or Algeria\(^{24}\). However levels measured in Polish MedWIs in 90-ies\(^{8}\) of last century were of a magnitude higher. Also levels of PCDD/Fs observed in bottom ashes from German MedWIs of higher class in a study from 2009\(^{25}\) were much higher. This suggests that major pathway of PCDD/Fs releases from small MedWIs is to air releases as stated in the Dioxin Toolkit\(^{10}\).

Table 5: Comparison of PCDD/Fs levels in bottom ash samples from MedWIs.

<table>
<thead>
<tr>
<th>Country</th>
<th>Year</th>
<th>PCDD/Fs</th>
<th>Class MedWI</th>
<th>Country</th>
<th>Year</th>
<th>PCDD/Fs</th>
<th>Class MedWI</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 African countries</td>
<td>2011-2019</td>
<td>347-2151</td>
<td>1 and 2</td>
<td>Germany(^{25})</td>
<td>2009</td>
<td>1160-19710</td>
<td>Class 4 (?)</td>
</tr>
<tr>
<td>Pakistan</td>
<td>2005</td>
<td>38-2105</td>
<td>1</td>
<td>Jordan(^{26})</td>
<td>2019</td>
<td>206-476</td>
<td>Class 2</td>
</tr>
<tr>
<td>Poland(^{8})</td>
<td>1998</td>
<td>7800-43000</td>
<td>2 and 3</td>
<td>Thailand(^{23})</td>
<td>2001</td>
<td>1390</td>
<td>Class 2</td>
</tr>
<tr>
<td>Vietnam(^{27})</td>
<td>2019</td>
<td>22.9-139</td>
<td>3</td>
<td>Algeria(^{24})</td>
<td>2018</td>
<td>1.68 - 878</td>
<td>Class 3</td>
</tr>
</tbody>
</table>

\(^{8}\) Poland\(^{8}\) refers to the period before 1998.\(^{23}\) Thailand\(^{23}\) refers to the period before 2001.\(^{24}\) Algeria\(^{24}\) refers to the period before 2009.
Levels of PCDD/Fs and dl PCBs in free-range chicken eggs from the vicinity of MedWI collected in 2018-2019 in 3 African countries was 5 - 105 times higher than levels in reference sample. With the exception of the eggs from Kumasi they also exceeded the EU limit (5 pg WHO-TEQ/g fat) for eggs as food by more than 2 - 105-fold. The EU limit would be exceeded also in eggs sampled around MedWIs in 2004-2005 (see Table 4). Some samples belong to those in which the very high levels of PCDD/Fs were measured in a global comparison, a level of 49 pg WHO-TEQ/g fat in eggs from Accra – hospital was 25-th highest globally.20

From comparison of patterns between egg and bottom ash samples in graphs at Figure 7 there is some similarity between eggs and bottom ash samples from Accra for PCDD/Fs while it is different for samples from Nkoltang showing less influence of bottom ash on PCDD/Fs profile in eggs. In all samples dl PCBs patterns (Figure 8) show a relation to waste incineration patterns in dl PCBs profile (presence of PCB 126 congener)20. However, eggs sample from Accra could be also influenced by a nearby e-waste site. Influence of waste incineration and other ash residues on PCDD/Fs levels in free-range chicken eggs was demonstrated already in previous studies,29,30

One of the previous studies in Africa very well described the practice of MedWIs bottom ash disposal: “The ash is carried in plastic buckets and dumped in open municipal waste sites ... In other cases and especially during the crop planting season, incinerator bottom ash is spread on agricultural farmlands as fertilizer supplement”31. This practice calls for setting strict limits for PCDD/Fs in wastes used on the land surface, and bottom ash from MedWIs should not be accessible to hens raised as local food. Results of some previous studies suggested to set a limit for untreated waste at levels of several tenths of pg TEQ/g dw as the maximum.32,33 Risks related to MedWIs on human health including POPs releases are underestimated. 33 The best way to avoid UPOPs releases is to prevent their creation by using alternative non-combustion techniques for medical wastes disposal.2,31,34,35 A stricter low POPs content level for PCDD/Fs + dl PCBs will also help regulate bottom ash from MedWIs.

5. Conclusions:
MedWIs are significant sources of environmental and food chain contamination by UPOPs, especially PCDD/Fs. The unregulated use of MedWI bottom ash appears to be a practice that contributes greatly to this contamination. This calls for setting a more stringent low level of POPs for PCDD/F and other limits on surface-applied wastes at the level of tenths of pg TEQ/g dm, as suggested by some of the previous studies. Application of the BAT/BEP Guidelines addressing technologies listed in Annex C to the Stockholm Convention especially to the medical waste incineration including replacement with non-combustion alternative techniques can also help to solve the situation.

6. Acknowledgments:
The study was financially supported by the Government of Sweden through IPEN, the Global Greengrants Fund, and the Sigrid Rausing Trust. "Waste transparently and without corruption", within the framework of which this abstract was created, was supported by the Open Society Fund under the Active Citizens Fund program.

7. References:


POP in Developing Countries

J. Okonkwo & B. Gevoa

1. Introduction:
Per- and polyfluorinated alkyl substances (PFASs) are anthropogenic chemicals which have been used in various industrial and commercial applications due to their distinct properties such as stain- and water-repellent abilities. PFASs are resistant to degradation, bioaccumulative and toxic. Wastewater treatment plants (WWTPs) play a role in the removal of contaminants from WWTP influent water. However, because conventional WWTPs are not designed to remove persistent contaminants such as PFASs, WWTP may account for the presence of PFASs in surface waters and groundwater. Where drinking water is abstracted from surface water and groundwater, the presence of PFASs in WWTPs, particularly in the effluent poses a great risk to the end-users. Therefore, this study aimed at investigating seasonal concentrations and patterns of PFASs in selected WWTPs. It is envisioned that this can lead to a better understanding of the fate of PFASs in wastewater treatment processes.

2. Materials and Methods:
WWTP samples were collected from the influents, primary settling tanks (PST), biological nutrient removal (BNR), secondary treatment tanks (SST) and the final effluents of 8 WWTPs during dry and wet seasons from four provinces in South Africa. A 1.2 mL of 50 mg/L of 21 individual native PFASs standards, 3 isotopically mass labelled (perfluoro-n-[1,2,3,4,5-13C5] octanoic acid (M2PFOA) were purchased. EPA Method 537.1 was adopted and followed for extraction, and 3 internal standards (perfluoro-n-[1,2-13C2] hexanoic acid (MPFHxA), perfluoro-n-[1,2-13C2] decanoic acid (MPFDA), and perfluoro-n-1,2-13C2 octanoic acid (M2PFOA) were purchased. EPA Method 537.1 was adopted and followed for extraction, clean-up and for analysis of PFASs. Multiple Reaction Monitoring (MRM) transitions and calibration were optimized, and PFASs were separated on a column with 2.6 um Polar C18 100 A LC Column 100 x 2.1 mm, at a temperature of 40 °C and injection volume was 10.00 µL. The flow rate was set at 0.30 mL/min. For method validation, blanks, influent, PST, BNR, SST and effluent samples were spiked with 21 PFASs, at low (5 ng/L), medium (800 ng/L) and high (1600 ng/L) concentrations, in triplicates. For further quality assurance and quality control, during every analysis, samples (200 mL), including blanks, were spiked with 100 µL of 200 ng/mL surrogates (MPFNA, MPFHxS and MPFUdA) to make a resulting concentration of 100 ng/L, before filtering and passing them through SPE (Solid Phase Extraction). Samples were filtered with a 0.45 µm and 0.2 µm glass fiber filters on a vacuum filtration unit before SPE. The cartridges were first conditioned with 5 mL of HPLC-grade methanol followed with 5 mL of ultra-pure water. Without allowing the cartridges to go dry, samples were passed through the cartridges at a flowrate of 0.5mL/min. After sample extraction, sample bottles were rinsed with 7 mL aliquots of ultra-pure water and each aliquot of ultra-pure water were drawn from the sample bottles and passed through the cartridge. The cartridges were then allowed to dry under a vacuum for an hour. PFASs were eluted with 10 mL of methanol under gravity in a dropwise manner. The eluents were concentrated to dryness under a gentle steam of nitrogen in heated water bath at temperatures between under 65 °C. The dried extracts were reconstituted in 1 mL of methanol and vortex for 1 minute. The reconstituted extract was then transferred to a 2 mL centrifuge tubes, the extract was then centrifuged for 5 minutes to separate possible fine particles suspended in the extract. A 950 µL of the extract and a 50.0 µL of 1000 ng/L of internal standard primary dilution standard (IS PDS) were added to an autosampler vial. A 10.00 µL of the samples were injected into the LC-MS/MS for analysis. Samples were run on an optimized internal standard calibration method

3. Results:
Recoveries were obtained for all surrogates at a range of 54.1-150 %, and 43.3-130 % for analytes. Low recoveries were exhibited by two long-chain PFASs in the biological nutrient removal (BNR) samples which had high suspended solid. Figure 1 shows mean concentrations of PFASs in WWTPs samples for dry and wet season. Concentrations ranged from 0.01-1268 ng/L and 0.01-4899 ng/L in dry and wet seasons, respectively. The concentrations where then log-transformed (Figure 2) to observe any existing relationships between the PFASs congeners and between the samples. Even in the absence of linear regression line, it can be observed that positive linear relationships exist for PFASs in different WWTPs samples in dry and wet seasons. However, PFASs in the BNR samples are distinctively on the same plane and separated from the other samples.
Figure 1: Concentrations of PFASs in WWTPs samples collected during wet and dry season. LP- Limpopo, MP- Mpumalanga, NC- Northern Cape and NW- North West provinces. WE- Effluent, WI- Influent, WB- Biofiltration Effluent, WP- PST, WS-SST, WSU- samples from the SST with effluent from UCT modified BNR and WU- UCT Modified BNR samples

PFASs in the PST samples were also spread on the same plane, but not distinctively separated from the PFASs in SST influent and effluent samples. PFASs in SST, influents and effluents samples showed an overlapping relationship. These observations are applicable to both dry and wet seasons. The US EPA advisory guideline of 70 ng/L for PFOA+PFOS was used as a limit for PFASs in the effluent samples, in the absence of any guideline on wastewater effluent.

Figure 2: Scatter plots of individual PFASs concentration (log_2 transformed) with their individual contributions across different influent, effluent, and unit processes for (a) dry and (b) wet seasons (Samples are presented by shapes while colours represent different PFASs congeners and the red horizontal line represents the US EPA advisory limit of 70 ng/L for PFOA+PFOS (n=27)

Few of the PFASs in the effluent exceeded this limit, including PFOA at concentrations of 1268 ng/L and 228 ng/L contributing to 91 % and 17 % in effluent samples from respective provinces during the dry season. Other PFASs which contributed to high concentrations in the effluent samples were FHET, FOET, PFBA, 8:2 FTS, PFHpS, PFBS and PFHxA. During wet season, 6:2 FTS exhibited the highest concentrations, of 4900 ng/L (66 %), 2351 ng/L (39 %), 1950 ng/L (53 %) and 1405 ng/L (29 %) in the influent, BNR, effluent and SST samples, respectively. PFOA and L-PFOS were among the PFASs which showed high concentrations in the effluent. PFOA was observed at 239 ng/L (6 %), 178 ng/L (5 %) and 156 ng/L (26 %), while L-PFOS exhibited 156 ng/L (10 %). Although most of the long-chain PFASs were observed at lower concentrations, L-PFDS was found at high concentrations of 171 ng/L (2 %) in the influent, 812 ng/L (19 %) and 425 ng/L (6 %) in the influent samples in the dry season. PFNA was also observed at 1498 ng/L (19 %) and 140 ng/L (2 %) in influent samples. During the dry season, PFNA contributed 85 % (112 ng/L) in the influent samples from one province. To further explore these patterns, correlations and PCA studies were carried out. During the dry season, significant positive correlations between PFCAs (p < 0.01) were observed for PFHxA with PFOA (r= 0.458), PFHpA (r=0.713), PFNA (r=0.700), PFOA (r=0.458) and PFBA (r=0.351), for PFHpA with PFBA (r=0.429) and PFOA (r=0.359), for PFNA with PFOA (r=0.673) and PFHpA (r=0.722). The correlation of PFSAs was only observed for L-PFHpS and
L-PFHxS ($p < 0.01, r=0.648$). Between the PFSAs and PFCAs, long-chain PFCAs were correlated with short-chain PFSAs; PFOA was significantly correlated ($p < 0.01$) with L-PFBS ($r=0.546$) while a significant negative correlation was observed for PFNA and L-PFHxS ($r=-0.471$). Fluorotelomers showed a significant correlation with each other and about 50% of both PFCAs and PFSAs. During the dry season, the ratio of PFBA/PFOA, which is used as domestic wastewater discharge was lower. Both PFOA and PFBA showed weak and negative correlation with one another, suggesting that the detection of the PFASs in the WWTPs may not solely be attributed to domestic discharge. During the wet season, significantly strong correlations ($p < 0.01$) were observed for long-chain PFASs, short-chain and all PFCAs. Fluorotelomers were mainly correlated with short-chain PFSAs. Similar to dry season samples, L-PFOS and PFOA were weakly and negatively correlated in wet season samples. A higher PFBA/PFOA ratio observed suggested that the detection of PFOA and PFBA may have originated from domestic discharge and other sources such as storm-water run-off during rainy seasons. These results show that the behaviour of most of the PFASs detected were dependent on one another, i.e., similar PFASs classes exhibited similar behaviour within the WWTPs. These results are corroborated by results obtained from PCA (Figures 3 and 4).

PCA of PFASs WWTPs samples was performed on log-transformed data where sample concentrations were >LOD, >LOQ and detection frequency >60%. In dry season (Figure 3(a)), the 1st and the 4th quadrants (anti-clockwise) had high loadings of PFBA/PFOA, 6:2 FTS, 8:2 FTS, FOET, FHET and PFPeA which are associated with a mixture of sources such as food packaging, precious metal coating, firefighting, coating textile and carpets cleaning agents, surfactants, and foam stabilizers in formation of AFFS. The 2nd and the 3rd quadrants indicated that the PFASs in the samples from Limpopo and North West were not as significant compared to the other provinces. The variability of BNR followed by SST samples seemed to be less compared to the influent and effluent samples (Figure 3(b)).

During the wet season (Figure 4(a)), the 1st and 4th quadrants (anticlockwise) were dominated by PFHxS, PFHxA, PFBA, PFPeA, PFHpA, 6:2 FTS, 8:2 FTS, FOET and FHET, while PFNA, L-PFBS, PFOA and FHEA, L-PFOS dominated the second quadrant. The wet season showed a wide range of PFASs contribution to PCA compared to the dry season. This could be as a result of rainfall. During the wet season, dilution and collection of contaminants from various sources into the WWTPs systems is expected. This could be due to the stormwater runoff. Rainfall has also been shown to influence the quality effluent of WWTPs. Interestingly, during wet season, most of the samples from NC, LP and NW closely clustered between quadrants 2 and 3 (anticlockwise), suggesting that they may be sharing similarities (Figure 4(a)). The variability of BNR and SST was similar to the observations in dry season as shown in Figure 4(b).
4. Discussion:
The concentration ranges of PFASs in WWTPs reported in this study are higher than those reported by Chen et al. Nguyen et al. reported PFOS (3.20 ng/L-92.0 ng/L) and PFHxA (13.0-20.0 ng/L) as dominant PFASs in the influent of a WWTP. However, in the present study, PFHxA (0.183-33.0 ng/L in dry season and <LOD-12.4 ng/L in wet season) was not the highest contributor in the influent, though their concentrations are comparable. Zhang et al. reported PFOS and PFOA as the dominant PFASs in the influent and effluent WWTPS samples, but the concentrations were lower than the levels observed in this study. The confidence ellipses on Figures 3(b) and 4(b) were able to segregate the different groups of samples within the WWTPS for both wet and dry season. High variability of PFASs was observed for samples collected during the dry season, while during the wet season, great variation was observed for NC-WE1, NC-W1I, NW-W2A and MP-W1I. A few studies on PFASs have also used PCA and cluster analysis to identify groupings of sampling sites in order to identify sources and their patterns. The present study was able to identify groupings on the location in dry season and on the type of unit processes in wet season.

5. Conclusions:
A wide range of PFASs was detected in WWTPs in the present study. The detection of long-chain PFASs, especially L-PFOS and PFOA, was an indication that these chemicals are still in use and/or have persisted from previous uses. Furthermore, the detection of PFASs in the effluent/within the WWTPs unit processes and at higher concentrations than the influent, is a confirmation that conventional WWTPs are unable to remove these chemicals and that there could be transformations within the WWTPs. Seasonal variation was also observed across the WWTPs, exhibiting different PFASs patterns. PCA analysis showed that PFASs patterns are different for most influent samples from different WWTPs, which suggested different sources of contaminations.

6. Acknowledgments:
The authors are indebted to the Water Research Commission of South Africa for financing this project.

7. References:
MON-PM2-C4  Assessment of Organophosphate Esters in Soil, Dust and Air Samples from an Electronic Waste Dumpsite in Lagos, Nigeria

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Introduction:
Improper recycling practices at informal electronic waste (e-waste) dumpsites in developing countries have led to substantial contamination with flame retardants (FRs), posing adverse public health impacts. E-waste, a significant source of persistent organic pollutants (POPs), contains hazardous materials like FRs that require special handling to avoid environmental contamination and harm to human health.

Organophosphate esters (OPEs) are extensively used as alternatives to legacy brominated flame retardants which have been listed by the Stockholm Convention as POPs. However, they have been linked to adverse effects such as carcinogenicity, neurotoxicity, endocrine and physiological effects, hepatoxicity and cytotoxicity (Wang et al., 2018). The occurrence of OPEs has been reported in several e-waste sites in developed countries (Li et al., 2019; Matsukami et al., 2015; Muenhor et al., 2017). The occurrence of OPEs in e-waste sites has been reported in developed countries, but there is limited information on the extent and impact in developing regions, particularly West Africa. Considering Nigeria’s status as the second-largest recipient of e-waste globally, with over four million computers and other e-waste categories received annually (Anselm et al., 2021), this knowledge gap is concerning.

Against this backdrop, this research evaluated the concentrations and relative abundance of OPEs in dust, soil and air samples in the vicinity of an e-waste dumpsite in Lagos, Nigeria; to guide on promoting responsible handling of e-waste.

Materials and Methods:
Standards of tris(2-carboxyethyl)phosphine (TCEP), Tris(1-chloro-2-propyl) phosphate (TCIPP), tris (1,3-dichloro-2-propyl) phosphate (TDCIPP), Tri-n-butyl phosphate (TNBP), Tri-phenyl phosphate (TPHP), Tri-2-ethylhexyl-diphenyl phosphate (EHDPP), as well as Isotopically labelled internal standards d₁₂-TCEP, d₁₅-TPHP, and d₁₅-TDCIPP (50 mg/mL in toluene) were purchased from Wellington laboratories, (Guelph, ON, Canada). The purity of all analytical standards was >98%. Indoor dust SRM 2585 and soil SRM 1944 were purchased from the US National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA). All solvents and reagents (HPLC grade) were obtained from Fisher Scientific (Loughborough, UK). Florisil® SPE cartridges were purchased from Biotage (Uppsala, Sweden).

Alaba International Market is located in Ojo Local Government Area, Lagos, Nigeria, with coordinates 6.4582°N and 3.3263°E. It is one of the most predominant and oldest business clusters renowned for collection, refurbishing and recycling of e-waste products (Manhart et al., 2011); and the largest of such site in Nigeria which engages in informal e-waste recycling.

A total of 36 samples consisting of 15 dust samples, 15 soil samples and 6 air samples were taken from the vicinity of the Alaba e-waste dumpsite in Lagos, Nigeria between June 2022 and August 2022; and investigated for the occurrence of seven OPEs. Samples were also taken from control sites, where there were no e-waste activities. Dust samples were collected using the methods of Iwegbue et al. (2019) and Nguyen et al. (2019); soil samples were obtained in accordance with Anselm et al. (2021) while air samples were collected using the method described by Tao et al. (2016). Briefly, the air samples were obtained using double bowl passive air samplers containing a polyurethane foam disk (PUF). The PUF in the samplers were deployed for 30 days intervals, and a sampling rate of 4m³/day was assumed for this study (Saini et al., 2020). On arrival at the laboratory, samples were stored in Ziploc bags at –20 °C prior to extraction.

Sample extraction and cleanup
The sample preparation and analysis methods applied were described in detail by Al-Omran et al. (2021). Samples were spiked with 50 ng of internal standards (d₁₂-TCEP, d₁₅-TPHP, and d₁₅-TDCIPP) and extracted using hexane: acetone (3:1 v/v). The pooled supernatant was evaporated to incipient dryness under a gentle nitrogen flow and extracts quantitatively transferred to a pre-cleaned Hypersep Florisil® cartridge. The second fraction, which contains the target OPEs was eluted with 10 mL of ethyl acetate. This was evaporated to incipient dryness and resolubilised in 100 µL of 500 pg/µL of PCB-62 in isooctane as recovery determination (or syringe) standard. The final cleaned extract was analysed using GC-EI/MS as reported previously (Gbadamosi et al., 2023). TCEP-d₁₂ was used to quantify TNBP, TCEP and TCIPP; TPHP-d₁₅ was used to quantify TBOEP, EHDPP, TMTMP and TPHP; TDCIPP-d₁₅ was used to quantify TDCIPP.
To evaluate method precision, replicate analyses were conducted of Standard Reference Materials (SRM 2585 and SRM 1944). One procedural blank and standard reference material were analysed for each batch of 10 samples. A 5-point calibration plot was constructed with OPE standard solutions in the concentration range 50 to 1000 pg/µL. For every ten samples, a 500-pg/µL OPE standard mixture was injected to check instrumental stability and calibration.

Descriptive statistics were calculated using Microsoft 365 Excel; the statistical distribution of the OPE concentrations was calculated using the Shapiro-Wilk and Kolmogorov-Smirnov normality tests; Analysis of variance (ANOVA) and Correlational Analysis (CA) were calculated with IBM SPSS 16.0; Kruskal-Wallis test and Mann Whitney test were carried out using Graph pad prism; while potential linear relationships between the variables were investigated using Spearman’s rank correlation.

Results:
The recoveries of internal standards in dust and soil samples were: 140 ± 25%, 57 ± 9.6% and 75 ± 15%; while those in air samples were: 140 ± 32%, 66 ± 27% and 78 ± 34% for d12-TCEP, d15-TPHP and d15-TDCIPP respectively. The 5-point calibration plot gave excellent linear response coefficients (r2 > 0.99) and the relative standard deviations of the relative response factors (RRFs) in the five calibration standards were below 10%.

The descriptive statistics for the concentrations of OPEs in dust, soil and air samples (arithmetic mean, median and range) are shown in Table 1.

Table 1: Concentrations of organophosphate esters in dust, soil and air samples around an e-waste dumpsite in Lagos, Nigeria (Concentrations of soil and dust in ng/g; Air in ng/m3).

<table>
<thead>
<tr>
<th>Matrix/ Parameter</th>
<th>TNBP</th>
<th>TCEP</th>
<th>TCIPP</th>
<th>EHDPP</th>
<th>TBOEP</th>
<th>TMTP</th>
<th>TDCIPP</th>
<th>TPHP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dust (n=15) Mean</td>
<td>510</td>
<td>720</td>
<td>3000</td>
<td>460</td>
<td>700</td>
<td>1000</td>
<td>360</td>
<td>2900</td>
</tr>
<tr>
<td>Median</td>
<td>340</td>
<td>430</td>
<td>2500</td>
<td>88</td>
<td>550</td>
<td>540</td>
<td>230</td>
<td>2600</td>
</tr>
<tr>
<td>DF%</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>95th Percentile</td>
<td>1600</td>
<td>2300</td>
<td>7200</td>
<td>1700</td>
<td>1800</td>
<td>3000</td>
<td>810</td>
<td>5700</td>
</tr>
<tr>
<td>Soil (n=15) Mean</td>
<td>2500</td>
<td>91</td>
<td>780</td>
<td>22</td>
<td>480</td>
<td>540</td>
<td>400</td>
<td>5600</td>
</tr>
<tr>
<td>Median</td>
<td>220</td>
<td>65</td>
<td>480</td>
<td>13</td>
<td>340</td>
<td>300</td>
<td>40</td>
<td>1900</td>
</tr>
<tr>
<td>DF%</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>95th Percentile</td>
<td>12000</td>
<td>2200</td>
<td>1900</td>
<td>60</td>
<td>1100</td>
<td>1700</td>
<td>2400</td>
<td>21000</td>
</tr>
<tr>
<td>Air (n=6) Mean</td>
<td>0.89</td>
<td>1.78</td>
<td>1.47</td>
<td>0.02</td>
<td>0.47</td>
<td>0.30</td>
<td>0.07</td>
<td>0.37</td>
</tr>
<tr>
<td>Median</td>
<td>0.59</td>
<td>1.48</td>
<td>0.96</td>
<td>0.02</td>
<td>0.31</td>
<td>0.23</td>
<td>0.06</td>
<td>0.33</td>
</tr>
<tr>
<td>DF%</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>95th Percentile</td>
<td>1.90</td>
<td>4.10</td>
<td>3.20</td>
<td>0.03</td>
<td>0.99</td>
<td>0.53</td>
<td>0.12</td>
<td>0.79</td>
</tr>
</tbody>
</table>

For dust samples, the mean and median concentrations of ∑8OPEs were 20000 ng/g and 5000 ng/g respectively. Among the analysed OPEs, TCIPP (3000 ng/g) had the highest mean concentration, followed by TPHP (2900 ng/g) and TMTP (1000 ng/g); while the lowest mean concentration was for TDCIPP (350 ng/g). The highest mean concentration of OPEs were found in storage shops, followed by dismantling, and then repair locations, with the lowest found in assembly sites. These differences were found to be significant (p<0.05) indicating significant variation in the magnitude of exposure of e-waste workers depending on which activity they were involved in.

In soil samples, the highest median concentration was of TPHP (5600 ng/g), followed by TNBP (2600 ng/g) and TCIPP (780 ng/g); while the lowest mean concentration was detected for EHDPP (22 ng/g). There was significant difference between the concentrations of OPEs in soil from areas conducting dismantling and burning.
In air samples, TCEP (1.80 ng/m³) was detected at the highest mean concentration, followed by TCIPP (1.5 ng/m³) and TNBP (0.89 ng/m³); while the lowest was EHDPP (0.02 ng/m³). There was no significant difference in all the target compounds amongst the indoor dismantling, outdoor dismantling and indoor repair and storage sites. However, the concentrations from outdoor samples were comparatively lower than the indoor air samples.

Discussion:
Similar to studies by Chen et al. (2020) in e-waste workshops, aryl- and alkyl-OPEs displayed the highest concentrations. The concentration pattern of dust samples in this study is similar to studies from e-waste sites in Qingyuan, South China (Zhou et al., 2022; Zheng et al., 2015) that reported TCIPP and TPHP as the most abundant analytes. Concentrations of OPEs in this study were lower than those reported in outdoor dust from a multiwaste recycling area in China (Wang et al., 2018), an e-waste dismantling facility in Ontario, Canada, (median 110,000 ng/g) (Stubbings et al., 2019); Guiyu, China (33,100 ng/g) (Zheng et al., 2015) and Southern China (25,000 ng/g) (He et al., 2015). However they exceeded those reported in Tianjin, China (11500 ng/g) (Wang et al., 2018) and Southern Thailand (average: 380 ng/g) (Muenhor et al., 2017).

In soil samples, the median concentration of OPEs in this study exceeds slightly that reported by Wang et al. (2018). The concentration pattern in the soil samples were similar to studies from e-waste dismantling sites in South China and Northern Vietnam where TPHP (10700 ng/g) and TCIPP (899 ng/g) displayed the highest OPFR concentrations (Ge et al., 2020; Matsukami et al., 2015). The predominance of TPHP might be due to the fact that it has found use as both a flame retardant and a plasticiser (Matsukami et al., 2015), has a strong affinity for soil particles (Wang et al., 2018), is often used in polyvinyl chloride, widely used in casings for household electronics; and has a long half-life in the environment; which consequently leads to its persistence in the environment (Ge et al., 2020).

In air samples, the abundance of TCEP and TCIPP in this study is similar to the study in a Canadian e-waste dismantling facility; where TCEP displayed the highest concentration 59 ng/m³, followed by TCIPP with a median of 50 ng/m³ (Nguyen et al., 2019). The high concentrations from outdoor samples compared to indoor air samples indicate a higher health risk to the e-waste workers who spend a larger proportion of their time indoor. It also indicates that the recycling facilities are the source of OPEs in the outdoor matrices.

Conclusions:
The results from this study suggest that informal recycling activities contribute significantly to OPE contamination of the surrounding environment. However, further studies are needed to explore overall occupational exposure in the e-waste site via human biomonitoring.
MON-PM2-C4 Assessment of Organophosphate Esters in Soil, Dust and Air Samples from an Electronic Waste Dumpsite in Lagos, Nigeria

Acknowledgements:
The authors acknowledge the Royal Society of Chemistry for the Analytical Chemistry Trust Fund Developing World Scholarship (ACTF-DWS Ref 22/600504/02) awarded to Moyofoluwa Ogungyemi, as well as the School of Geography, Earth & Environmental Sciences, University of Birmingham, UK for their research support.

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Introduction: Clean air, water, and soil are critical to the sustainability of life on Earth. Today, there is a growing interest in environmental screening for harmful substances in both outdoor and indoor settings. Chronic exposure to hazardous materials via inhalation or ingestion during work, rest, or play is detrimental to human health. Therefore, it is extremely important to screen for toxic materials in the environment regularly. Unfortunately, monitoring hazardous chemicals such as persistent organic pollutants (POPs) is challenging due to a large number of different compounds and the sheer complexity of environmental matrices.

Materials and Methods: The goal of this study was to develop a methodology for the comprehensive screening of environmental samples for both legacy and emerging pollutants. This included the application of effective sample preparation and introduction techniques, as well as the design of general data acquisition and processing strategies for complex samples. Pretreatment of samples was minimized to avoid the loss of any constituents. Instead, small quantities of the sample were introduced into analytical instrumentation through 1) Simple extraction, concentration, and injection, 2) Thermal desorption, and/or pyrolysis. This was followed by analysis using two-dimensional gas chromatography, a novel multi-mode source, and high-resolution time-of-flight mass spectrometry (GCxGC-MMS-HRTOFMS). Instrument software provided complete acquisition control, in addition to automated instrument optimization for a seamless transition between the three different ionization modes: 1) Electron Ionization (EI), 2) Positive Chemical Ionization (PCI), and 3) Negative Chemical Ionization (NCI).

Results: The methodology provided comprehensive data with exceptional peak alignment between ionization modes, improved S/N, and an increase in the total number of annotated compounds. EI data were utilized to annotate compounds through untargeted data processing which consisted of spectral similarity searches of large databases, and formula determinations using high-resolution accurate mass molecular, and fragment ions. PCI and NCI spectra provided complementary molecular formula information and increased confidence in compound characterization. Advanced software tools (e.g., scaled mass defect & RDBE plots) simplified retrospective analysis of the comprehensive data for emerging pollutants. The analyses resulted in the annotation of polymeric markers, polymer additives, heterocyclic compounds, aromatics, polyaromatics, polyfluorinated compounds, and bisphenols.

Discussion and Conclusion: The non-targeted methods facilitated the comprehensive screening of environmental samples. This approach is an alternative to targeted methods that often focus on specific classes of pollutants. The rich GCxGC-MMS-HRTOFMS mass spectra serve as "historical data archives" that can be retrospectively analyzed for unknown and/or emerging pollutants in the environment. These pollutants are often absent from current databases but can be characterized using complementary EI-, and CI-HRTOFMS data and modern spectral analysis software tools. The described methodology is very valuable for the effective monitoring of air, water, and soil.
Progress in Methods for POPs Analysis
G. Eppe & G. Hunt

MON-PM2-D2 Sliding windows in ion mobility (SWIM): a new approach to increase the separation power in trapped ion mobility-mass spectrometry hyphenated with chromatography

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Introduction:
In recent years, the separation capability of commercially available IMS instruments has improved drastically. Advanced linear IMS technologies, including the TIMS, cyclic cTWIMS, and SLIM, have demonstrated impressive resolving powers exceeding 300. However, achieving such high resolving power typically requires extended measurement times or analysis of ions over a restricted ion mobility range, which can pose significant challenges when coupling the IMS-MS instrument with front-end chromatography and analyzing compounds with a wide range of collision cross section (CCS) values. This can be particularly problematic in the analysis of environmental pollutants, where a broad range of CCS values are typically present.

Materials and Methods:
We have developed a novel approach for improving the resolving power of trapped ion mobility spectrometry (TIMS) when coupled with gas chromatography, called “Sliding Windows in Ion Mobility” (SWIM). This method was applied to a challenging mixture of 175 persistent organic pollutants (POPs), including halogenated dioxins, halogenated biphenyls, and polybrominated diphenyl ethers (PBDEs). Our measurements were performed using a Bruker TIMS TOFpro II mass spectrometer equipped with a GC-APCI II source for sample separation and ionization, prior to TIMS-MS analysis.

Results:
The resolving power in TIMS has been demonstrated theoretically and experimentally to be dependent on several parameters, including the scan rate $\beta_v$, which represents the rate at which voltage is decreased during the elution step. When the analysis is performed over a shorter ion mobility range ($\Delta V_{ramp}$) and/or with a longer analysis time ($t_{ramp}$), the scan rate decreases ($\beta_v = \Delta V_{ramp}/t_{ramp}$), resulting in improved resolving power for the analytes of interest. However, in standard TIMS operation, the ion mobility range must be large enough to trap and analyze all the compounds of interest, while the analysis time must be short enough to cope with the time scale of the front-end separation technique. This limitation impedes the use of slow ramp speed and significantly restricts the achievable resolving power in chromatography hyphenated TIMS applications.

Our SWIM approach builds upon the exceptional ability of TIMS to selectively trap ions within a specific range of ion mobilities. Contrary to the standard TIMS mode which employs a broad and constant IM analysis range, the SWIM mode uses narrow and mobile ion mobility windows that are continuously scanned and adapted to the ion mobility range and elution time of the targeted analytes throughout the gas chromatographic run. For example, in the analysis of POPs, the ion mobility windows were initially set to target early eluting, low halogenation degree pollutants with the lowest CCS values, and then gradually increased to analyze pollutants with increasing halogenation degree and higher CCS values.

Discussion and Conclusion:
The use of narrow ion mobility ranges in SWIM mode led to significantly improved resolving power (~40%) compared to the standard mode. This improved the separation of several critical GC coeluting isobaric and isomeric pairs, such as the positional PCB isomers 84 and 90-101 which were partially separated in standard TIMS mode but baseline separated in SWIM mode. Overall, although all coeluting isobaric pairs could be separated in the ion mobility dimension, most coeluting isomeric pairs remained unresolved, despite the higher resolving power provided by the SWIM approach. Nevertheless, these results demonstrate the great potential of the integration of high resolution ion mobility within GC-MS systems for the monitoring of halogenated POPs.

Acknowledgments: This publication is supported by the French Community of Belgium through the funding of a FRIA grant (FC 47331). The authors would also like to thank the Belgian Federal Public Service of Health, Food Chain Safety and Environment through the contract RT22/06 TEQFOOD.

References:
MON-PM2-D3 Reducing the Environmental Impact of Multi-Class Semivolatile Organic Compound Analysis

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Introduction: The analysis of multi-class, semivolatile organic compounds (SVOCs) in environmental matrices is a common high throughput analysis in many labs. Therefore, modernizing SVOC analytical methods has the potential to significantly reduce the environmental impact that labs themselves have on the environment. An important aspect of reducing the environmental impact of SVOC methods is reducing solvent consumption associated with sample preparation. Improvements to the sensitivity of the analytical technique facilitate the goal of reduction of solvent use. In contrast to electron ionization (EI) gas chromatography coupled to atmospheric pressure chemical ionization (GC-APCI) produces intense molecular ions for most analytes. When combined with tandem quadrupole MS/MS, the sensitivity of GC-APCI combined with MS/MS directly contributes to the improved environmental sustainability of SVOC analytical methods. Furthermore, GC-APCI is readily adaptable to the use of nitrogen as an alternative carrier gas to helium. This also reduces the footprint of the overall method since helium is a non-renewable resource and a by-product of petroleum production. Previous work on different applications, such as pesticides in food matrices, has demonstrated elements of these factors on prior models of tandem quadrupole\textsuperscript{1}. In this work we demonstrate that the same benefits are achievable for SVOCs on a new generation of mass spectrometer with reduced electric consumption and heat output.

Materials and Methods: A previously developed helium carrier gas based SVOC method\textsuperscript{2} was adapted for the use of nitrogen carrier gas by scaling the column dimensions to achieve equivalent chromatographic separation and runtime. A 10:1 split injection was used with the injection port at 310°C. The GC-APCI source was operated in charge exchange (aka dry source) mode with nitrogen as the chemical ionization (CI) reagent gas. The tandem quadrupole mass spectrometer was operated in positive ion mode. Multiple MRM transitions were developed for each analyte using a standard solution of 76 compounds plus 6 internal standards (IS). Nitrogen was also used as the collision induced dissociation (CID) gas, as opposed to the argon CID gas used in earlier work, allowing the entire system to run on a single gas supply. Additional filters were added to the house nitrogen supply from a large centralized dewar in order to ensure the highest purity supply of nitrogen since with this configuration it plays a role in separation, ionization and fragmentation. Following method development on the latest model tandem quadrupole the dry source method was transferred to a previous generation tandem quadrupole. That system was operated with helium carrier gas and argon CID gas using the same MRM transitions as the all-nitrogen system in order to generate comparative data.

Results: Initial collision energy (CE) values for the system using argon CID gas were estimated using a method translation guide that calls for the nitrogen CEs to be multiplied by 0.75 for use with argon. A subset of transitions was then systematically evaluated at different steps of CE to verify the effectiveness of this guidance. All but one CE value agreed within 2eV with the outlier being 5eV at 25eV. This was determined to be suitable agreement to allow sensitivity, linearity and reproducibility assessments to be carried out.

Both the all-nitrogen system and the system using helium carrier achieved equivalent chromatographic separations and sensitivity as shown in the examples below. Across all analytes separations were functionally equivalent and sensitivity was within a factor of two of the same with the nitrogen system having the advantage for some analytes and the helium system for others.

Figure 1. Benzo(a)anthracene/chrysene separation, upper He carrier, lower N\textsubscript{2} carrier
Dilution series across the range of 1 to 1000 ppb (in -vial conc.) were analyzed with >85% of analytes achieving a minimum $r^2$ value of >0.990. The %RSDs for all ISs were <15% for data from both systems.

**Discussion and Conclusion:** The equivalency of the data across the two configurations demonstrates the feasibility of both for performing quantification of multi-class SVOCs extracted from environmental matrices at sensitivity levels compatible with the scaling of sample preparation to reduce solvent consumption while also using a split injection to reduce matrix loading on the column. With respect to the use of nitrogen carrier gas this is in good agreement with prior work on pesticides. With respect to the use of nitrogen CID gas, previous work by others has been used as a guidance for adapting methods between the different configurations. The current work then provides additional support to the idea that the tandem quadrupole MS configuration effectively decouples the ionization mode and source design from the CID process. Plans to extend this work will include the analysis of extracts of environmentally relevant matrices and the evaluation of long-term reproducibility and robustness of the technique.

**References:**
Progress in Methods for POPs Analysis

MON-PM2-D4  Comparative Analysis of Nitrogen Carrier to Helium for APCI Gas Chromatography in Food Matrices

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Introduction: Gas chromatography, coupled to tandem quadrupole mass spectrometry (GC-MS/MS), is a powerful analytical technique used for the detection and quantification of persistent organic pollutants in food and environmental samples. Traditionally using Helium as a carrier gas; recent difficulty sourcing helium worldwide has led to significant price increases and demand for alternative carrier gases for gas chromatography. Nitrogen is readily available, relatively inexpensive at the necessary purity and both unreactive and safe as compared to other options. This presentation will demonstrate the ease of transfer of GC-MS/MS methods, originally performed using helium carrier gas, to nitrogen. Specifically, the GC-MS/MS used in these studies incorporates an atmospheric pressure ionization (API) source, allowing for additional flexibility in gas choice and flow rates. Additionally, the atmospheric pressure ionization GC-MS/MS (APGC) enhances sensitivity relative to traditional vacuum source GC-MS/MS, resulting in the ability to modify sample preparation techniques without a negative impact in detection or reporting limits.

Materials and Methods: An analytical method containing over 200 target pesticides was evaluated on the APGC Xevo™ TQ-S micro GC-MS/MS system (Waters Corporation) using both helium and nitrogen carrier gases. The evaluated samples of cucumber extract were prepared using modified Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) sample preparation methodology. Extracts of infant food (cottage pie) were also prepared for pesticide analysis using Nitrogen as the carrier to show the equivalent performance of each gas when using APGC, simply by scaling the analytical method parameters including column dimensions and gas flow. The GC columns were: Rxi-SVOCms column – 30m x 0.25mm x 0.25µm for helium analysis, – 20m x 0.15mm x 0.15µm for nitrogen analysis (Restek). A commercially-available 203 GC amenable pesticide mix target compound analytical reference material for the 2 food matrices was used (Restek). Cucumber and infant food using QuEChERS extraction (Waters Corporation) into acetonitrile was analyzed with an in-house MS/MS method on the APGC Xevo™ TQ-S micro.

Results: The switch of carrier gas was an easy transition as scaling the column dimensions along with a small amount of method development allows for the retention times to be matched from one carrier gas to another. This allowed many of the method files used for identification and quantification to be kept the same for each type of carrier gas. The nitrogen analysis showed equivalent calibration coefficients for the historically “difficult” compounds as well as equivalent resolution of critical pairs. Both carrier gases run on APGC Xevo™ TQ-S micro show superior sensitivity to an EI based system and demonstrated consistent performance when using either helium or nitrogen as carrier.

Discussion and Conclusion: Utilizing nitrogen as a carrier gas should yield a significant advantage to labs who have observed budgetary impact from the rising cost and scarcity of helium. The improved sensitivity of APGC when compared to EI systems opens the possibility of using split injections and/or lower amounts of sample which would further benefit overall laboratory efficiency through decreased cost of consumables and reagents. Finally, Nitrogen is not observed to alter the mass spectral properties for the target compounds, which can be an observed difficulty if using a reactive carrier gas (hydrogen, for example).
MON-PM2-E1  In vitro hepatic metabolism of polychlorinated biphenyls with different chlorine-substituted structures in rats and humans: kinetics, metabolism, and potential nuclear receptor affinities

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Introduction: Polychlorinated biphenyls (PCBs) and the biotransformation of PCBs have gained considerable attention from environmental chemists and toxicologists because of their prevalence in the environment and potential adverse effects in humans and wildlife (Jepson and Law 2016). Different hydrogen substitution structures may affect the metabolic activities and pathways of PCBs. However, previous research mainly investigated specific PCB congeners in animal species and lacked the health risk assessment of PCBs and their products. In the current study, different chlorine-substituted structures (PCB 77/110/136/174) were selected to explore the hepatic metabolic characteristics of PCBs in rats (RLM) and humans (HLM). The potential nuclear receptor affinities of PCBs and PCB metabolites were investigated to predict their potential biological effects.

Materials and Methods: Metabolic substrates (including PCB 77, PCB 110, PCB 136, and PCB 174), surrogate standards (PCB 30, PCB 65, and PCB 204 for PCBs and 4'-OH-PCB 159 for OH-PCBs), internal standards (PCB 24, PCB 82, and PCB 198 for PCBS and 4-MeO-PCB 72 for OH-PCBS), and other standards used for daily correction (4'-MeO-PCB101, 3-MeO-PCB118, 3-MeO-PCB138, and 3-MeO-PCB182) were purchased from Accustandard Inc. (USA). Moreover, we employed molecular docking, a computational and high throughput method, to have an initial understanding of the interactions between tested PCBs or OH-PCBs and nuclear receptors (NRs) to analyze the difference in potential effects between OH-PCBs and their parent PCBs.

Results: The rate constants ($k_{obs}$) of PCBs showed variations in the order patterns for the HLM (PCB 136 > PCB 110 > PCB 174 > PCB 77) and RLM (PCB 110 > PCB 136 > PCB 174 > PCB 77). However, studied PCBs showed similar metabolite profiles and enantioselective of PCBs between HLM and RLM. The Mono-OH-PCBs were the major metabolites of PCB 77/174, whereas mono-OH- and di-OH-PCBs were the major metabolites of PCB 110/136 for the HLM and RLM, indicating that OH-PCBs could be further oxidized. Enantiomeric enrichment of (−)-PCB 136 and (+)-PCB 174 was observed in microsomal metabolism. Moreover, the inflection point of the enantiomer fraction for PCB 136 metabolized by the HLM was observed in the present study. Furthermore, molecular docking results demonstrated the relatively high affinity between PCBs (or OH-PCBs) and certain nuclear receptors, indicating that abnormal metabolic enzyme expression and endocrine disruption occur in PCB-exposed humans.

Discussion and Conclusion: The species-dependent liver microsome metabolism capacity of PCBs was consistent with previous studies, which might be attributed to the differences in the CYP-450 enzyme profile. The different OH-PCB profiles between our study and previous studies may result from the profiles difference of the CYP-450 enzymes and also these differences might be related to the differing incubation conditions, such as reaction times and the concentrations of substrates, NADPH, and liver microsomes. Specifically, the enantiomer fractions variations for PCB 136 with reaction time exhibited a parabolic shape in the HLM system, implying competitive metabolism between individual atropisomers of chiral PCBs. Moreover, selected PCBs and their potential hydroxylated products exhibited relatively high affinities for human aryl hydrocarbon receptor, constitutive androstane receptor, pregnane X receptor, and androgen receptor, indicating that abnormal metabolic enzyme expression (mediated by NRs) and endocrine disruption occur in PCB-exposed humans. As a discrepancy existed between parent PCBs and OH-PCBs in the binding affinities to NRs, the effect of metabolism on the exerted biological impact needs to be studied further.

Acknowledgments: This work was supported by the National Natural Science Foundation of China (Nos. 42277242 and 41977306), Guangzhou Science and Technology Project (No. 202002030134)

References:
From October 2021, they were fed with contaminated hay [PCDD/F concentration in the range 2.3-12.7 ng TEQ kg\(^{-1}\) dry matter] to PCDD/Fs (i.e., mostly raised on pasture paddocks where soil PCDD/F concentrations are the highest in Lausanne). Recruited from the Lausanne sheep herd. An “experimental: EXP” group (n = 5) originated from the folk the most exposed to PCDD/Fs (i.e., most often raised on pasture paddocks where soil PCDD/F concentrations are the highest in Lausanne).

1. Introduction:
Production systems for foods of animal origin (e.g., eggs, milk, meat) have faced several contamination incidents with persistent organic pollutants (POPs, e.g., polychlorinated dibenzo-p-dioxins and furans, PCDD/Fs) over the last decades. Some well-known livestock contamination incidents were caused by waste incineration, with subsequent PCDD/F atmospheric deposition and persistence in the surrounding topsoils. A recent case was revealed in Lausanne, Switzerland in 2021 with soil PCDD/F contamination from an old municipal waste incinerator. Although the contaminated soils are mainly located in urban areas, sheep are kept in these areas for meat production. In order to ensure food safety, predicting the transfer of PCDD/Fs from soil to sheep meat and estimating the time needed for depuration of formerly exposed herds is essential. Unfortunately, the use of static feed-to-food transfer factors (e.g., bioconcentration factors) is not applicable to a diffuse source of exposure from soil, as it requires a “steady-state” condition, which is reached only after long-term constant exposure. In Lausanne, such conditions are never met, as soil PCDD/F levels vary widely among the grassland paddocks where the sheep transitorily graze. In this context, dynamic physiologically-based toxicokinetic (PBTK) models are the most appropriate tools to quantify soil to meat accumulation and to predict depuration kinetics. The aim of this study was to develop and assess the performance of a PBTK model describing the fate of PCDD/Fs in lactating ewe using in vivo toxicokinetic data for model development. The case study in Lausanne represented a unique opportunity to develop and test such a model.

2. Materials and Methods:
Animal experimentation. The experiment was approved (n°VD3750) by the committee on animal experimentation of canton Vaud, Switzerland and took place from April to November 2022. Nine multiparous gestating “Roux du Valais” ewes were recruited from the Lausanne sheep herd. An “experimental: EXP” group (n = 5) originated from the folk the most exposed to PCDD/Fs (i.e., most often raised on pasture paddocks where soil PCDD/F concentrations are the highest in Lausanne). From October 2021, they were fed with contaminated hay [PCDD/F concentration in the range 2.3-12.7 ng TEQ kg\(^{-1}\) dry matter (DM)] harvested in Lausanne. On the 1\(^{st}\) April, 2022 (day 0 of experiment), that is 29 days after lambing, EXP ewes initiated a depuration phase after being switched to non-contaminated hay (0.04 ng TEQ/kg DM) harvested at Agroscope Posieux, which they received until the end of the experiment (day 188). The “control: CTL” group (n = 4) belonged to a folk that mostly pastured on low- to moderately-contaminated areas, and received low-contaminated hay from Lausanne (0.15 ng TEQ kg\(^{-1}\) DM) from October 2021 until January 2022, and further the non-contaminated hay from Agroscope. Ewes were fed at 8:00 am hay ad libitum and 350 g DM d\(^{-1}\) per ewe (280 g after day 140) of a concentrate feed and the same amount of pelleted whole maize plant. They were housed in a free-stall barn and had free access to water and a mineral salt block. Lambs were raised with their mothers until weaning at 92 days old (day 63 of experiment), and further ewes were non-lactating and non-gestating. Slaughter took place at the end of the experiment (day 188).

Sub-samples of feeds were harvested once a week and further pooled over 1 to 3 months periods for PCDD/F and nutrient analyses (DM, ashes, crude fibers, crude proteins and ether-extracted fat, see Driesen et al.\(^{1}\) for details). Measurements and sampling on EXP ewes were performed at depuration days 0 (start of the depuration period, 29 days after lambing), 32 (61 days after lambing), 60 (89 days after lambing: time of weaning/dry-off), 130 and 188 (slaughter). The CTL ewes lambed on average 35 days after the EXP ewes and the measurement and sampling schedules (at days 0, 60 and 188 only) were adjusted accordingly. At each sampling, ewes were weighed, scored for fat cover and sampled for milk (by hand-milking at days 0, 32 and 60) and subcutaneous sternal adipose tissue (biopsy under sedation and local anesthesia, except for post mortem sampling at day 188). At slaughter, liver was additionally sampled, before the empty body (full body without digesta) was weighed and its lipid mass determined by dissection, grading and chemical analysis. Analysis of the seventeen 2,3,7,8-chloro-substituted PCDD/F congeners concentrations was performed as described in Driesen et al.\(^{4}\). In brief, lipids were extracted by Soxhlet or liquid-liquid extraction for milk, before addition of \(^{13}\)C labeled internal standards, and clean-up on acidic and base silica, alumina and activated carbon (EZprep Fluid Management Systems). Quantification was further achieved by atmospheric-pressure gas-chromatography – mass spectrometry (APGC-MS, Waters...
Xevo-TQ XS) in the daughter ion scan (MS-MS) by isotope dilution technique. Development of the PBTK model. Model implementation was performed on Vensim® Professional software (7.3.5, Ventana Systems, Harvard, USA). The ewe PBTK model describes the PCDD/F absorption, distribution, metabolism and excretion (ADME) and is based on former models developed for lactating cows and growing cattle (Figure 1). The fate of individual PCDD/F congeners is described within digestive contents and six body compartments. Contaminant intake flows from soil, feed and digestive contents into the digestive tract, where it is either excreted back to the digestive contents or distributed between liver, adipose tissues (first to blood-perfused, later to deep compartments), udder milk and the other tissues. Metabolic clearance is located in the liver. The 11 blue arrows in Figure 1 represent advective flows among compartments.

\[
\text{Adv. Flow Conti} \rightarrow \text{j} \quad (\text{ng d}^{-1}) = Q_{i \rightarrow j} \quad (\text{kg d}^{-1}) \times A_i \quad (\text{ng}) / M_i \quad (\text{kg}) / P_i, \quad (1)
\]

where \( Q_{i \rightarrow j} \) is the rate of digesta transit, blood perfusion (gathered from MacLachlan) or milk excretion from i to j, \( A_i \) is the contaminant amount in i and \( M_i \) the mass of i. The partition coefficient \( P_i \) reflects the tissue-blood ratio of the contaminant concentration at equilibrium, and is only used for the flows from tissue compartments back to blood. The \( P_i \) was initially fixed to the tissue-blood ratio of total lipid concentrations, assuming that non-polar lipophilic contaminants diffuse almost exclusively and uniformly into total lipids. Deviations have been reported for the observed milk to blood lipid-normalized ratio (< 1) of highly chlorinated congeners in cows and the specific PCDD/F liver sequestration (high liver to adipose tissue ratio) observed in lambs. To compensate these, the correction factors \( \text{Corr}_i \) were introduced, which multiply the respective \( P_i \). \( \text{Corr}_i \) is the observed liver to adipose tissue ratio (day 188 at slaughter for EXP and CTL ewes, n = 9; Table 1). The four yellow arrows in Figure 1 are diffusive flows, where the contaminant crosses the interface between two compartments by passive diffusion along the concentration gradient. Those include the digestive tract / blood interface for absorption and reverse non-biliary excretion, and the perfused / deep adipose tissues interface for representing the late (re)distribution pattern of lipophilic contaminants in adipose tissues. For both interfaces, the contaminant serially crosses a water phase (unstirred water layer surrounding the microvilli of the intestinal wall or adipocyte cytosol) and a lipid phase (lipid bilayer of enterocyte or adipocyte membranes).

\[
\text{Diff. Flow Conti} \rightarrow \text{j} \quad (\text{ng d}^{-1}) = \left[ A_i \quad (\text{ng}) / V_{ij} \quad (\text{m}^3 \text{ lipids}) \right] / \left[ (K_{ow} \quad / \quad Q_{\text{water}} \quad / \quad j \quad (\text{m}^3 \text{ d}^{-1})) + (1 \quad / \quad Q_{\text{lip}} \quad / \quad j \quad (\text{m}^3 \text{ d}^{-1})) \right], \quad (2)
\]

where \( V_{ij} \) is the volume of lipids in i, \( K_{ow} \) the partition coefficient between octanol and water (Table 1) that converts a lipid- to a water-based concentration, and \( Q_{\text{water}} \) and \( Q_{\text{lip}} \) the diffusive parameters across the water and lipid phases at the interface between i and j, respectively. For the digestive tract / blood interface, \( Q \) parameters were adjusted by dividing by 7.4 the values fitted in lactating cow by MacLachlan. Such 7.4-fold factor corresponds to the ratio of empty intestines mean weight in adult cows from Driesen et al. to the one in ewes of the present study.

Liver is assumed to be the only site for contaminant degradation (i.e., metabolism; purple arrow, Figure 1).

\[
\text{Degr. Flow Cont}_{\text{liver}} \quad (\text{ng d}^{-1}) = A_{\text{liver}} \quad (\text{ng}) \times k_{met} \quad (d^{-1}), \quad (3)
\]

where \( k_{met} \) is the first-order metabolism rate constant. \( k_{met} \) was the only model parameter fitted for single PCDD/F congeners against the kinetics of milk and adipose tissue concentrations of the EXP ewes observed in vivo, using the Payoff procedure of Vensim. This calibration was performed for 12 congeners only (Table 1), the 5 remaining being most of the time recorded at levels lower than the limits of detection.

In order to resolve the rest of the equations of the PBTK model, the kinetics in fresh and lipid masses in every compartments should be described. The feed, digesta and feces flows were estimated by using the feed lipid content, digestibility and rumen fill unit (determined from nutrient contents).
The hay intake was estimated using the INRA feeding system for sheep\(^1\), with body weight and fatness score, lactation stage, hay fill unit, concentrate intake, lamb growing rate and ambient temperature as predictive variates. Similarly, milk yield and milk fat yield were estimated from lactation stage and lamb growth rate\(^1\). Empty body weight and lipid mass were estimated from body weight and fatness score, using predictive equations set at the time of slaughter (day 188, \(n = 9\)). Body fresh and lipid masses were allocated to the specific body compartments according to allometric relationships established for cows\(^2\).

After full implementation of the model, the EXP and CTL ewe cases were simulated using the estimated physiological traits, feed and initial day 0 milk and adipose tissue PCDD/F concentrations as input data and parameters.

Calculations and statistical analyses. In vivo data of physiological traits and PCDD/F concentrations in milk and adipose tissue were analyzed using the MIXED procedure of SAS (version 9.4, SAS institute, Cary, USA). The model for repeated measures included day, treatment (EXP vs. CTL) and their interaction as fixed effect, and ewe as a random effect using a spatial power covariance structure. A mono-exponential depuration model (\(C_t = C_0 \times \exp (-k \times \text{day})\)) was further adjusted to the least square means of PCDD/F concentrations in milk and adipose tissue of EXP ewes, using the NLIN procedure of SAS. The same statistical analysis was performed on the simulated milk and adipose tissue concentrations extracted at the same days. For adipose tissue, the concentration at day 188 was excluded, as it was not different (\(P > 0.10\)) from day 130 and led to worse adjustment (lower \(R^2\) and higher RMSE). The depuration half-life was calculated as \(\ln(2) / k\).

### 3. Results:

**Physiological traits.** Physiological traits are presented in Figure 2. Body weight of EXP ewes increased from 50 kg at day 0 to 59 kg at day 188 (\(P < 0.001\)). Conversely, CTL ewes had a stable body weight along the experiment (in average 64 kg), which was higher than the one of EXP ewes at days 0 and 32 (\(P < 0.05\)) and their interaction as fixed effect, and ewe as a random effect using a spatial power covariance structure. A mono-exponential depuration model (\(C_t = C_0 \times \exp (-k \times \text{day})\)) was further adjusted to the least square means of PCDD/F concentrations in milk and adipose tissue of EXP ewes, using the NLIN procedure of SAS. The same statistical analysis was performed on the simulated milk and adipose tissue concentrations extracted at the same days. For adipose tissue, the concentration at day 188 was excluded, as it was not different (\(P > 0.10\)) from day 130 and led to worse adjustment (lower \(R^2\) and higher RMSE). The depuration half-life was calculated as \(\ln(2) / k\).

### Table 1

<table>
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<tr>
<th>Congener</th>
<th>(\log K_{ow})</th>
<th>(C_{orr} P_{milk})</th>
<th>(C_{orr} P_{liver kmet})</th>
<th>(k_{day})</th>
<th>(C_{observed})</th>
<th>(C_{simulated})</th>
<th>(C_{observed})</th>
<th>(C_{simulated})</th>
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<td>0.8</td>
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1. The 12 congeners were consistently over limit of detection in milk and adipose tissue.
2. In vivo observed or PBTK model simulated data at days of depuration 0, 32 and 60 for milk, and 0, 32, 60 and 130 for sternal adipose tissue were adjusted to a mono-exponential model (\(C_t = C_0 \times \exp (-k \times \text{day})\)), where the half-life equals \(\ln(2) / k\).
MON-PM2-E2  In Vivo and In Silico Physiologically-Based Toxicokinetic Investigations of PCDD/F Depuration in Ewes

At the end of the exposure period (depuration day 0), the highest concentrations were recorded in EXP ewes with slightly higher values in milk (27.2 pg TEQ g⁻¹ lipids) than in adipose tissue (23.2 pg TEQ g⁻¹ lipids, Figure 3). Both were around 10-fold higher than the EU sheep milk and meat maximum level (ML of 2.0 and 2.5 pg WHO-TEQ PCDD/Fs g⁻¹ lipids, respectively, EU 1259/2011 regulation). Parallel exponential decays for the two penta-congeners and the sum TEQ were observed in both milk and adipose tissue. At the end of lactation (day 60), this led to 5.5- and 2.9-fold decreases compared to day 0 (P < 0.01) for the sum TEQ in milk and adipose tissue, respectively. Accordingly, higher concentrations were recorded in adipose tissue (8.1) than in milk (5.0 pg TEQ g⁻¹ lipids) at the end of lactation. After weaning, adipose tissue concentrations continued decreasing (P < 0.001), reaching 2.0 pg TEQ g⁻¹ lipids at day 130, compliant with the ML. Concentration did not change further significantly (P = 0.44) until day 188 (1.6 pg TEQ g⁻¹ lipids), which was still higher than in CTL ewes (0.8 pg TEQ g⁻¹ lipids, P = 0.02). When compared to penta-chlorinated congeners, a similar exponential decay characterized the depuration of 1,2,3,4,6,7,8-HpCDD in milk, whereas its adipose tissue concentration decreased more slowly and in a linear fashion (Figure 3). Apparent monoeXponential depuration half-lives in milk and adipose tissue are presented in Table 1. Shorter half-lives were consistently recorded in milk than in adipose tissue (2.3-fold difference in average, n = 11 congeners), with exception of OCDD, for which a longer half-life was seen in milk than in adipose tissue. Accordingly for the sum TEQ, half-lives were of 21 d in milk and 41 d in adipose tissue.

PBTK model evaluation. Dotted lines in Figure 3 illustrate the model simulated kinetics in milk and adipose tissue PCDD/F concentrations. In EXP ewes, which served as a model calibration dataset, simulations were in remarkable agreement with in vivo data, with under 1.3-fold divergence between observed and simulated values for penta-chlorinated congeners and sum TEQ. Satisfactory accuracy and precision were confirmed when simulations were plotted against observations for all twelve congeners. Mean positive bias of +0.17 pg g⁻¹ lipids (+6% of the mean of observed values), RMSE of 0.43 pg g⁻¹ and R² equals to 0.93 were calculated for milk (n = 24), with slightly poorer performance for adipose tissue [negative bias of -0.36 pg g⁻¹ (-11%), RMSE of 0.69 pg g⁻¹ and R² = 0.97 (n = 48)]. Model performance was comparable when CTL ewe’s data served as an external dataset, with mean bias = -0.02 (-5%) and -0.15 (-28%) pg g⁻¹; RMSE = 0.21 and 0.31 pg g⁻¹; and R² = 0.72 and 0.70; for milk (n = 12) and adipose tissue (n = 24), respectively. Accordingly, depuration half-lives derived from model simulations were in remarkable agreement with the ones from in vivo observations, with a consistent 8% average overestimation of milk half-lives and a 2% average underestimation of the adipose tissue half-lives.

4. Discussion:
This work confirms that it is feasible to depurate ewes with an initial PCDD/F level around 10-fold the EU ML over a time period of less than four months. The PCDD/F concentration recorded in vivo in adipose tissue was compliant with the ML after 130 days of depuration, whereas according to the PBTK model simulations, such a level may be achieved after around 100 days.

Ewes were depurated from day 0 (EXP, n = 5) or served as non-contaminated control (CTL, n = 4). Weaning took place at day 63.
During lactation (days 0 to 60), depuration probably occurred mainly through excretion of PCDD/Fs via milk lipids. Accordingly, estimated total lipids excreted through milk were 4.6 kg, which is 1.3-fold higher than estimate of body lipid mass during lactation. After weaning (days 60 to 188), ewe adipose tissue concentrations continued to decrease steadily. Over such a period, excretion is limited to fecal output, and the dilution effect from increasing body lipids (3 kg at day 60 to 11 kg at day 188) likely played a major role in the decline of adipose tissue concentration. A similar dilution effect was outlined in growing cattle for PCBs\(^{18}\), as well as in calves for PCDD/Fs and PCBs\(^{6}\). The reverse was also observed in depurated non-lactating ewes, that is, an increase in adipose tissue TCDD and PCB 126 and 153 concentrations, due to a sharp decrease in body lipid mass following undernutrition\(^{19}\).

Bi-exponential decay models are commonly used to describe the depuration of POPs in milk\(^{6,20}\). As no measurements were performed between days 0 and 30, only a mono-exponential model was used ad-hoc in the present study. In order to perform inter-species comparison, cow milk data at days 0, 35 and 62 of depuration\(^4\) were also fitted to a mono-exponential model. The resulting apparent mono-exponential milk half-lives for the 12 PCDD/Fs congeners investigated were on average 2.2-fold longer in cows\(^4\) than in EXP ewes. Such higher depuration potential in ovine than in bovine, may be partly due to the fact that EXP ewes excreted more milk fat per body lipid mass than cows in Driesen et al.\(^4\), making milk a more effective excretion pathway in ewes. Alternatively, this may point a higher ovine hepatic metabolic rate. Indeed, compared to cows and calves\(^8\), a specific liver sequestration (i.e., high liver to fat ratio) was recorded in ewe (present study) as well as in lamb\(^{12}\), a process suggested to be central for hepatic clearance\(^8\).

Comparison of milk and adipose tissue depuration half-lives revealed that the latter were consistently longer. This is most probably explained by a late (re)distribution and equilibrium between adipose tissue and blood, as already noticed in non-lactating cows\(^{15}\). This process was captured by the PBTK model, thanks to the implementation of diffusive flows at the interface between perfused and deep adipose tissue compartments according to the fugacity concept\(^{15}\).

5. Conclusions:
PBTK models describing the fate of POPs in lactating\(^6,11,15,20,21\) or growing cattle\(^{10}\) have been proposed, but models for sheep are scarce. The predictive ability of livestock PBTK models for POPs has also been rarely quantified\(^22\). Thus, the ewe PBTK model introduced in the present study is a step forward in the in silico description of POP fate into ruminant milk and meat production systems across species, breeds, physiological status and feeding systems. Ongoing developments include extension for other POPs (e.g., PCBs) and additional validation for the accumulation phase. The additional development of a PBTK model describing POP fate in growing lambs and its coupling to the current ewe model is also required to describe transgenerational transfer in suckling sheep systems. Those steps will allow the delivery of integrative in silico tools for risk assessors and managers, and ultimately contribute to the chemical safety of animal products.

6. Acknowledgments:
This project was co-funded by the “Direction générale de l’environnement (DGE)” of canton Vaud (Lausanne, Switzerland) and the Federal Food Safety and Veterinary Office (FSVO, Bern, Switzerland) under the contract ProxyPOP (n°4.23.03). The authors warmly thank G. Guex and the team of the Lausanne municipality for the conduction of the sheep experiment; P. Mermoud (Proviande, Bern, Switzerland) for fat score measurements, B. Hayoz, C. Driesen, B. Egger, F. Sansonnens and I. Morel (Agroscope) for their valuable support in ewe measurements, sampling and slaughter; the teams of the Feed Chemistry and Animal Biology Research Groups (Agroscope) for proximate chemical analyses; and K. Lehner, P. Rickenbach, M. Rüegg and E. Trivelin (Empa) for PCDD/F analyses.

7. References:
MON-PM2-E2  In Vivo and In Silico Physiologically-Based Toxicokinetic Investigations of PCDD/F Depuration in Ewes


MON-PM2-E3  Toxicokinetic modeling of the transfer of polychlorinated biphenyls (PCBs), dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) into milk of high-yielding cows during negative and positive energy balance

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Introduction: Polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs or “dioxins”) are environmentally persistent substances that accumulate in the tissues of exposed animals and humans. Foods of animal origin represent the main route of human exposure to dioxins and PCBs. In this regard, milk is of particular interest, since milk is a prominent nourishment for humans and animals. At the same time, lactation is the main elimination route of dioxins and PCBs for dairy cows. It is therefore of great importance to describe the transfer of dioxins and PCBs from feed to cows’ milk. The aim of our study was to derive transfer parameters for a large number of dioxins and PCBs to be used for risk analysis. Furthermore, we aimed to determine whether the metabolic state of the animal has a significant influence on the transfer.

Materials and Methods: To better understand the transfer of dioxins and PCBs into cows’ milk in different metabolic states, a feeding study was conducted. In this feeding study, German Holstein cows (n=5) were dosed with 17 dioxins and 18 PCBs for 28 consecutive days at the beginning of lactation, when the animals were in the negative energy balance (NEB) phase and again for 28 consecutive days during their latter positive energy balance (PEB) phase. Throughout the lactation period, milk and blood samples were taken and chemically analyzed for the dioxins and PCB concentrations. To evaluate the derived data, a physiologically-based toxicokinetic (PBTK) model was developed and fitted to the data, deriving separate parameter sets for the NEB and PEB phase. The model consists of 3 compartments: a central blood compartment and two storage compartments divided into fast and slowly perfused. The model was used to derive various transfer parameters, such as steady-state transfer rate (TR) and half-lives for both NEB and PEB phases. The NEB and PEB transfer parameters were statistically compared to determine if there was a significant difference between these two phases.

Results: The model developed here was able to describe the concentration-time curve of dioxins and PCBs in milk and blood reasonably well. For all but 3 of the dioxins and PCBs investigated, transfer parameters could be derived, including congeners for which transfer parameters had never previously been reported. Testing for differences between the NEB and PEB phases showed that the TR was statistically significantly higher during the NEB phase than during the PEB phase (p<10⁻⁰) when all congeners were considered simultaneously. However, on a congener-specific level, this difference was only significant for 8 out of 32 congeners. Furthermore, when analyzing the mean residence time of the congeners in the blood compartment, it was found that they remained longer in the blood compartment during the PEB phase than during the NEB phase (p<10⁻¹² for a statistical test considering all congeners simultaneously). On a congener-specific level, this is significant for 4 out of 32 congeners. In this context, the time to reach 90% of the steady-state concentration in milk (Tss) was also estimated; it was concluded that Tss with 100 or more days for some congeners is significantly longer than the length of most experimental feeding study designs. No significant differences in Tss were observed between the NEB and PEB phases. Similarly, the derived half-lives showed no significant difference between the NEB and PEB phases.

Discussion and Conclusion: In order to accurately predict the transfer of dioxins and PCBs, the metabolic status of the animal must be taken into account, as it influences the transfer of dioxins and PCBs from feed to milk. Furthermore, future experimental study designs that aim to predict the steady-state TR in milk should take into account that it takes several months for the animal to reach near steady-state conditions. Therefore, either longer exposure times or a mathematical extrapolation technique are required.
Analysis of Per- and Polyfluoroalkyl substances in Edible Fish tissue using Enhanced matrix removal lipid extraction and LC/MS/MS detection

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Introduction:
The primary source of chronic exposures to per- and polyfluoroalkyl substances (PFAS) in humans is through the ingestion of contaminated foods and drinking water, with fish and other seafood being a major contributor. Therefore, the analysis of PFAS in food is an emerging topic. In various regions of the world regulations for PFAS in food are already in place or being drafted. Additionally, in the USA oral minimal risk levels (MRL) have been derived for 5 PFAS components. Therefore, there is an urgent need for reliable and easy-to-use analytical methods. We describe an analytical method for edible fish tissue, quantifying 25 different PFAS components with easy-to-use sample preparation and excellent detection limits. Additionally, we describe the applicability of the method in 140 fish samples from different species.

Materials and Methods:
2 gram of fish tissue was weighed and each samples was homogenized using formic acid and acetonitrile. After centrifugation the samples were extracted using enhanced matrix removal lipids SPE extraction. Internal standards were added to the clean extract. For determination of recovery, accuracy and precision, experiments were preformed using surrogate standards added after weighing the 2 grams of fish tissue. The extracts were analyzed on a binary LC system that was adapted to reduce the PFAS system background. Analysis was performed using a C18 column using gradient elution of ammonium acetate and acetonitrile. Injection to injection runtime is 31 mins. 20 µL of the extract were injected on a triple quadrupole MS operated in negative ionization mode with dynamic multiple reaction monitoring (dMRM).

For this study fish samples were collected at 25 different locations in the Tampa Bay, FL USA area. In total 140 individual fish samples were measured from 24 different fish species.

Results:
The average surrogate recovery in fish samples was outstanding and ranged from 101 to 114% and RSDs from 12 to 16% and the overall surrogate recoveries in method blanks ranged from 71 to 129%. These results were well within the typical accepted criteria of 70 to 130% accuracy and less than 30% RSD, indicating this is a robust and reliable extraction and analysis procedure. Chromatographic peak shapes were very good and calibration curves used were from 1 to 1,000 ng/L. All 140 collected samples were positive for PFAS. Concentrations varied largely between the different collection sites. With the more coastal regions having the lowest (average 2,030 ng/kg w/w) and areas being more enclosed by land having the highest (average 15,600 ng/kg w/w) PFAS concentrations in edible fish muscle tissue. The fish profiles were generally dominated by PFOS (68 +/- 17%). Additionally, large difference between fish species and PFAS concentrations were observed.

Discussion and Conclusion:
A robust and reliable extraction procedure for the analysis of 25 PFAS components in fish muscle tissue has been developed. The method has immediately been applied on 140 real fish muscle samples, and those samples were from 24 different species, demonstrating the versatility of the method to different sample types. Those samples clearly show different amounts of PFAS components per location. At some locations concentrations are rather high demonstrating the urgency of measuring PFAS in fish. We will now continue to develop methods for other types of food.

References:
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Introduction:
Per- and polyfluorinated alkyl substances (PFAS) are one of the most commonly discussed and researched environmental contaminants. This is due in part to how widespread and impactful PFAS contamination has become, but also from increasing media awareness. Initially, most focus was on monitoring PFAS in water, mainly drinking water, but the prominence of these compounds in everyday items have expanded analysis to other matrices such as food, cosmetics, and human biofluids. As more research is focused on PFAS exposure and impacts, analytical techniques have been advancing as well to help progress research and monitoring efforts.

One of the constantly changing areas in PFAS analysis is the list of PFAS compounds that are being targeted for. One trend impacting this is the movement of manufacturers from using long chain PFAS to short chain and ultra-short chain PFAS. As the chain length shortens, chromatography can become more complicated, not only from a perspective of the analytical column the sample is separated on, but also regarding the functionality of the isolator column that is crucial to delaying PFAS interference from the LC system. These compounds require advancements in chromatographic techniques to properly detect and quantify them in samples. A mixed mode column using both reverse phase and anion exchange chemistry was investigated and will be presented as a solution to the short chain PFAS challenge.

Materials and Methods:
A new mixed mode column (XBridge™ Premier BEH™ C18 AX Column) was evaluated as both an isolator column and an analytical column to provide better retention of short chain and ultra-short chain PFAS. Samples tested were provided by US EPA Region 5 and included water collected from a metal finisher, a hospital, bus washing station, a powerplant, a pulp and paper mill, ground water, surface water, wastewater influent, wastewater effluent, and landfill leachate. All samples were prepared following the ASTM 8421 protocol. Briefly, 5 mL of sample were diluted with methanol, syringe filtered, and acidified prior to analysis using LC-MS/MS utilizing the mixed mode column.

Results:
The mixed mode C18 AX column provides a significant increase in retention of the short and ultra-short chain PFAS through utilization of the ionic head groups of the PFAS compounds that are retained using the anion exchange mechanism, rather than just relying on the reverse phase retention mechanism used by the hydrophobic C-F chains. The increase in retention allows for the ultra-short chain PFAS (< C4) to be included in a single injection of a typical suite of PFAS when utilizing it as an analytical column, but it also is beneficial as an isolator column to delay common contaminants like PFBA (C4 carboxylate) that typically can co-elute with the analytical peak when using just a reverse phase only column, such as a C18.

A suitable gradient was developed to take advantage of the C18 AX chemistry by increasing the pH over the course of the gradient. This allows the column to be used to analyze a large suite of PFAS chemistry classes, spanning the C-F chain lengths of C2 through C14 (and above). Eleven different water samples were run using this method, which contained a variety of PFAS. The samples were evaluated using a previous method that was not capable of retaining < C4 PFAS and therefore these compounds were not previously screened for. Analysis on the mixed mode column resulted in detection of three PFAS (TFA, PFPrA, and PFPrS) previously undetected in the samples.

Discussion and Conclusion:
Utilization of a column for PFAS analysis that is more specific to the chemistry of the compounds has allowed the expansion of the typical suite of PFAS possible in one method to now include the ultra-short chain compounds, such as TFA, PFPrA, and PFPrS. Typical methods that do not include the ultra-short chains can significantly underestimate the level of PFAS quantified in a sample, as demonstrated by the samples evaluated in this study. Additionally, utilization of this column chemistry provides better delay of chromatographic interferences, therefore providing potential increases in method sensitivity, and most importantly more accurate and confident sample quantitation.

Acknowledgments:
The authors would like to acknowledge Lawrence Zintek at the US EPA Region 5 for providing samples to use for this evaluation.

References:
TUE-AM-A3  New Reference Materials for PFAS

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Introduction: Standard Reference Materials (SRMs) are homogeneous, well-characterized materials that are used to validate measurements and improve the quality of analytical data (https://www.nist.gov/srm/srm-definitions). The National Institute of Standards and Technology (NIST) has a wide range of SRMs that have values assigned for legacy organic pollutants. These SRMs can serve as target materials for method development and quality assurance/quality control (QA/QC) of chemical measurements. As a unique class of organic contaminants, per and polyfluoroalkyl substances (PFAS) present measurement challenges to the environmental analytical community that can affect the accuracy and precision of quantitative measurements. Currently, NIST has reference materials (human serum, house dust, sludge, fish tissue, etc.) with values of PFAS measured in them; however, there are relevant gaps in the NIST library of reference materials. A major gap is reference materials of high level PFAS, including technical mixtures, like aqueous film-forming foam (AFFF) and PFAS impacted soils/sediments. Working with partners, NIST has been expanding the list of reference materials for PFAS measurements.

Materials and Methods: NIST has been working with the Department of Defense to acquire legacy AFFFs and PFAS impacted soils to create six new reference materials value assigned for PFAS. In addition to NIST measuring PFAS in the materials, NIST has conducted an interlaboratory study to see the utility of the candidate AFFF reference materials.

Results: Four AFFF reference materials have been measured for PFAS and are available to aid in quantitative and qualitative measurements of PFAS (Reiner et al., 2023). Interlaboratory results from the AFFF interlaboratory assessment showed poor agreement among laboratories and the need for reference materials for the validation and quality assurance of laboratory measurements of PFAS. The two candidate soil materials are currently being homogenized at NIST. After the homogenization process, these materials will be measured and certified for PFAS at NIST.

Discussion and Conclusion: Four AFFF reference materials are now available from NIST (https://shop.nist.gov/). The values and uncertainties of the PFAS quantified in the AFFF reference materials were results of two or more independent measurement methods. These reference materials are useful to the global analytical community and can serve as materials for quality assurance measurements and method development.

Acknowledgments: Creation of the four AFFF reference materials was funded by the Strategic Environmental Research and Development Program (SERDP), project number ER18-1664.

References:
**Introduction:** Per- and Polyfluoroalkyl Substances (PFAS) contain a perfluorinated or polyfluorinated carbon chain moiety such as F(CF2)n- or F(CF2)n-(C2H4)n. In recent years there has been increasing concern over the levels of these chemicals, (e.g., PFOS (perfluoro sulfonate), and PFOA (perfluoro-octanoic acid)) in the global environment because of their fate and possible adverse effects. PFOS are subject to varying but increasing levels of control in various countries. In the United States the Environmental Protection Agency (US EPA) has released various methods for extraction and analysis of PFAS compounds such as method 533, 537.1 and 1633 (draft). Solid Phase Extraction (SPE) has become a well-accepted technique for these kinds of analyses. In this study we describe a fully automated system that was made specifically for PFAS extraction. Primarily effective at reducing background contamination, extraction and concentration of aqueous samples takes less than two hours. The application described here is especially aimed at wastewater analysis following draft method 1633 (1).

**Material and Methods:** Six synthetic wastewater samples (500 mL) were spiked with 50 ppt native PFAS standards and relevant internals. Sample bottles were loaded onto the system and Weak Anion Exchange (WAX) cartridges were installed in each of the six positions. Rinse bottles are automatically filled during the entire procedure to rinse the empty sample jars. Positive pressure (nitrogen) is used for pumping solvents and mixes through the system and vacuum is used to load the samples. The cartridges were conditioned with 15 mL of 1% methanolic ammonium hydroxide and with 5 mL of 0.3M formic acid. Samples were loaded across the cartridges at 5-10 mL/min (~ 8-inch Hg). The sample bottles were then rinsed with 5 mL reagent water (twice) followed by 5 mL of 1:1 0.1M formic acid/methanol and these rinses were loaded onto the system. After 15 sec of drying, the rinse bottles were filled automatically with 5 mL of 1% methanolic ammonium hydroxide, used to spray the walls of the empty sample jars. The rinses were sent across the cartridges, the elutes were collected in polypropylene tubes and as per the method no further concentration was carried out. Extracts were collected Relevant standards were added prior to LC/MS analysis.

**Results:** A total of 40 native PFAS compounds were analyzed using EPA method 1633. All recoveries were 88% or higher with RSDs (%) all < 10%. All PFAS recoveries were within the acceptance windows (different for each compound) required by the method. Labeled deuterated and 13C surrogates had average recoveries of 102% with an average RSD of 10%. Total run time of the automated system is < 90 min. The automated system produces very good recoveries with low standard deviations. An important problem with ground and wastewater extraction is the presence of particulate matter which can easily plug up cartridges. Use of plastic filtration wool in the barrel of the cartridges can eliminate this problem. In this work no clogging of cartridges was observed.

**Conclusions:** Wastewater samples can be analyzed with USEPA method 1633 using a fully automated system that can process six samples in less than 1.5 hours. Because the system is closed native background cross contamination is very low. Excellent data is obtained with this system that is modular in nature and fast and reliable.

**References:** (1) Method 1633: Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS. U.S. Environmental Protection Agency Office of Water (4303T) Office of Science and Technology Engineering and Analysis Division 1200 Pennsylvania Avenue, NW Washington, DC 20460. December 2022.
To see, or not to see: the direct total oxidizable precursor assay as a tool to identify PFAS hotspots in German rivers

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Introduction: The group of per- and polyfluoroalkyl substances (PFAS) comprises several thousand substances. The ever growing number is a major challenge for their chemical analysis. One analytical approach to overcome the complexity of PFAS are sum parameters, such as the total oxidizable precursor (TOP) assay.

Materials and Methods: In this study, we conducted a spatial monitoring study with more than 210 suspended particulate matter (SPM) and sediment samples from rivers and lakes from Germany and The Netherlands to analyze spatial differences and identify contamination hotspots. All samples were analyzed by target analysis and a modified TOP assay, the so called direct TOP (dTOP) assay, in which a small amount of sample is completely digested, converting previously unmeasurable precursors to measurable perfluorinated compounds.

Results: The analysis revealed substantial differences between the different water bodies in both, the level and type of contamination. The ∑PFAS concentrations ranged from <0.5 to 53.1 µg/kg dry weight (dw) in the target analysis and from <1.0 to 337 µg/kg in the dTOP assay. The levels of perfluoroalkyl acids (PFAA) were substantially higher in the dTOP assay compared to the target analysis demonstrating the significant presence of unidentified precursors in the environment. As a simplistic approach to identify hotspots of PFAS contamination, the 90th percentiles (P90) for target analysis (P90_target: 7.11 µg/kg dw) and dTOP assay (P90_dTOP: 79.1 µg/kg dw), respectively, were used as thresholds. Both methods identified 17 hotspots, but only six of the sampling sites were consistently identified as hotspots by both methods. Thus, the majority of hotspots identified with the dTOP assay was overlooked by the classical target analysis.

Discussion and Conclusion: The results of this study demonstrate the ubiquitous burden of PFAS in German rivers especially by unknown precursors. Only some of the hotspots identified in this study were (publicly) known before. At many of the hotspots and other sampling sites, however, the source of the PFAS contamination remains unknown and must be elucidated in the future to prevent further discharge into the environment. The data set will serve as a baseline to assess the effectiveness of oncoming regulatory actions for PFAS.

Acknowledgments: The authors thank the German Federal States, the German Federal Institute of Hydrology and the Dutch Authority Rijkswaterstaat for providing the samples and for their support.
Advances in the (Bio)Remediation of POPs

J. He & S. Lomnicki

TUE-AM-B2  Application of biofilm-based inoculum delivery system for organohalide respiration of polychlorinated biphenyls (PCBs) in sediments

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Introduction: Removal of polychlorinated biphenyls (PCBs) from contaminated sediments is a priority because of their ability to enter the food chain and due to their toxicity. Commonly adopted remedies include dredging and capping which are associated with challenges including disruption of existing habitat and high cost. While in situ microbial degradation of PCBs represents an improvement, previous attempts have failed because of PCB stability, low bioavailability, low abundance and activity of indigenous PCB-degrading microorganisms. The high efficiency of activated carbon (AC) and other sorptive substrates to quickly sorb PCBs from sediments has been demonstrated. Co-localizing PCB-degrading microbes onto surfaces of sorptive particles as biofilms and utilization as a delivery system provides a novel approach to address PCB contamination. In this project, several biofilm-covered adsorbent materials including AC were evaluated for enhancement of PCB dechlorination in sediment. Dechlorination kinetics, biofilm colonization and biofilm growth on various carrier materials was also investigated.

Materials and Methods: Biofilms of anaerobic Dehalobium chlorocoercia DF1 (DF1) enrichment cultures from wastewater and soil containing organohalide respiring bacteria as well as aerobic Paraburkholderia xenovorans strain LB400 (LB400) were formed on sorptive materials. The materials consisted both of activated carbons and biochars based on plant and animal waste products such as coconut shell, pine wood, acai and bone. Mature biofilms were inoculated into PCB contaminated sediment mesocosms. The formation of biofilm on the sorptive materials was quantified using culture-based methods, molecular and microscopic approaches. Experiments were further carried out examining PCB dechlorination with DF1 which was scaled up, grown and maintained using tandem 20 L bioreactors. Biofilm formation on various carrier materials was investigated in PCB 61-spiked microcosms. In addition, sorption kinetics were investigated and the biological rate of dechlorination of PCB 61 to PCB 23 was measured in sediment microcosms. Techniques employed in this work include DNA extraction with specific 16S rDNA primers, polymerase chain reaction (PCR), quantitative-PCR (qPCR), confocal laser scanning microscopy (CLSM), scanning electron microscopy (SEM), gas chromatography coupled to electron capture detection (GC/ECD) and mass selective detection (GC/MS).

Results: Bioaugmentation of PCB contaminated Grasse River sediment with dechlorinating biofilm covering GAC showed increased Aroclor 1248 degradation. The results also showed that the augmentation benefitted other bacterial groups that were not directly linked to PCB degradation thus the delivery system could provide additional benefits compared to the objective. To assess the mechanism behind the increased PCB dechlorination, the importance of the material characteristics for selected sorptive and non-sorptive materials was evaluated. One of the main findings was that sorption of PCBs to activated carbon was the driving force behind the biofilm formation by Dehalobium chlorocoercia DF-1. DF1 formed a patchy biofilm ranging in cellular biomass thickness from 3.9 to 6.7 µm with an average of 4.6 ± 0.87 µm, while the biofilm coverage area varied from 5.5% (sand) to 20.2% (activated carbon) indicating a preference for highly sorptive carrier materials. Quantification of DF1 bacteria in the biofilms showed abundances from 1.2 to 15.3 x10^9 bacteria per g material. These results show that biofilm-based inoculum for bioaugmentation of weathered PCBs in sediment can be an efficient approach.

Discussion and Conclusion: Results of this work demonstrate that it is possible to predict PCB microbial dechlorination in sediments provided accurate measurement of the aqueous phase dechlorination kinetics and knowledge of site-specific partitioning characteristics. In addition, experimental results show DF1 and LB400 biofilm-based inoculum was more effective at PCB degradation than liquid inoculum. Furthermore, DF1 biofilms preferentially formed on highly sorptive carrier materials including AC purporting biofilm-coated AC as a promising bioaugmentation approach to remedy PCB-impacted sediments. The results provide the basis for future research to determine if biological activated carbon is an effective remedy strategy in subsurface systems.

Acknowledgments: This study was supported by the Department of Defense Strategic Environmental Research and Development Program, SERDP project ER-2135 to Dr. Birthe V. Kjellerup.
TUE-AM-B3  Dehalogenation of hexachlorocyclohexane isomers by iron and iron sulfide micro and nanoparticles. Study of reaction mechanism with stable carbon isotopes and pH variations

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Introduction: For many decades, remediation of chlorinated organics contamination was focused on their anaerobic and aerobic biodegradation, which might be accompanied by isotope fractionation of carbon (13C/12C). Nevertheless, the abiotic reductive dehalogenation by zero-valent iron nanoparticles1 and iron minerals2 has attracted considerable attention within the research community, but mostly on halogenated solvents and fuel additives and much less on chlorinated pesticides. In this study, we explored the dehalogenation of hexachlorocyclohexanes (HCHs) isomers by both zero-valent iron and iron sulfide particles of different sizes (µm and nm), and investigated the associated reaction mechanisms.

Materials and Methods: All degradation experiments were carried out in 250 mL anaerobic bottles screwed gas-tight by butyl/silicon septa. The dehalogenation experiments with zero-valent iron particles were performed for α-HCH alone (in duplicates at pH 7.3), as well for mixture of α, β, γ and δ-HCH at neutral pH values, on an incubator at 125 rpm and 30ºC. The dehalogenation experiments with FeS nanoparticles were performed for α-HCH and δ-hexachlorocyclohexane (HCH) only, under similar experimental conditions, while using a wide range of pH values (2.4 to 11.8). The concentrations of HCH isomers and their degradation products were investigated by GC-MS and GC-MS-MS measurements, while their isotope compositions were investigated by gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS).

Results: In the experiments involving iron particles, the primary intermediate degradation products of HCHs were identified as tetrachlorocyclohexanes (TCCHs), while the main final degradation product was identified as monochlorobenzene (MCB). During the dehalogenation experiment of α-HCH by iron particles, the initial concentration of α-HCH was found to be 22.86 ± 1.35 µM, which decreased to 0.38 ± 0.06 µM after 6 days of degradation, indicating a first-order kinetics. In contrast to the degradation process using zero-valent iron particles, the degradation of HCHs through FeS nanoparticles follows a different pathway involving the formation of pentachlorocyclohexanes (PCCHs) as the main intermediate degradation products. For the dehalogenation of α-HCH by FeS, the results of three different experiments showed that the apparent rate constants during dehalogenation increased with pH. Regardless of the pH used, the 1,2,4-trichlorobenzene (1,2,4-TCB), 1,2-dichlorobenzene (1,2-DCB), and benzene were the main degradation products of α-HCH and FeS nanoparticles, without the accumulation of MCB. An enrichment factor (ε) of -4.7 ± 1.3 % was calculated for α-HCH, which is equivalent to an apparent kinetic isotope effect (AKIE) value of 1.029 ± 0.008 for dehydrohalogenation, and of 1.014 ± 0.004 for dihaloelimination, respectively.

Discussion and Conclusion: In contrast to previous studies utilizing zero-valent iron nanoparticles4, which suggested that intermediates such as tetrachlorocyclohexanes could further transform via the putative dichlorocyclohexadiene (DCCH) to benzene, this study primarily observed the formation of MCB as the main degradation product of α-HCH. The larger particle sizes of the Fe used in this study may have led to a slower reduction reaction of DCCH to benzene, facilitating its spontaneous decomposition into MCB. Additionally, the isotope fractionation observed in the experiments with FeS nanoparticles indicates that abiotic isotope fractionation by FeS should be considered in anoxic sediments and aquifers contaminated with HCH isomers when high concentrations of FeS are present in such environments.

Acknowledgments: This research was performed within the project 584 PED/2022-“New eco-nano-technologies for the elimination of halogenated organic compounds from wastewater using advanced oxidation and reduction processes and anaerobic biodegradation processes”, funded by UEFISCDI, Romania.

References:
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Introduction: Per and polyfluoralkyl substances (PFAS) are a group of fluorinated compounds, which are persistent and mobile in the environment, and have been linked to several adverse health effects. One mode of environmental spread of PFAS is infiltration into groundwater from polluted sites and further spread with the groundwater flow. A method to stop this contamination plume is with pump-and-treat techniques. The EU LIFE SOuRCE project aims to demonstrate two treatment trains for pump-and-treat solutions for PFAS contaminated groundwater, one at a landfill in Sweden and one at a site in Spain where PFAS containing firefighting foams have been used. An important factor to take into account when considering pump and treat is the soil water partitioning coefficient ($K_d$) and the sorption kinetics in the aquifer. A high $K_d$ value could be detrimental to the process by retaining the pollutant in the aquifer during pumping, the same is true for fast adsorption kinetics and slow desorption kinetics. Experiments are therefore conducted to determine these properties.

Method: Drill cores have been collected from the aquifer soil matrix while establishing groundwater wells at the two sites in Sweden and Spain. PFAS were analyzed in soil and groundwater from the aquifer and field $K_d$'s were determined at the Swedish site. Desorption and adsorption tests were conducted in laboratory experiments using the collected aquifer soil material from the two sites, 4 locations at the Swedish site and 2 locations at the Spanish site. In the desorption experiments, soil from the contaminated aquifers were shaken with ultra-pure water in a liquid to solid (L/S) ratio of 2 L/kg. Samples were taken out at 4 times (0.5, 4, 168, and 336 h) in order to establish desorption kinetics as well as $K_d$ values. During the adsorption experiments soil samples from all tested aquifers were shaken with ultrapure water spiked with a mixture of 10 PFAS, using 4 different time points (0.5, 4, 24 and 168 h).

Results: The desorption kinetics were rapid, reaching steady-state concentration at 0.5 h for all samples. The $K_d$ values were low for all samples, being <1 for all compounds above the reporting limit in the solid face: perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorobutanesulfonic acid (PFBS), perfluorohexanesulfonic acid (PFHxS), Perfluorooctane Sulfonic Acid (PFOS) and 6:2 Fluorotelomer sulfonic acid (6:2FTS). This was also true for the field $K_d$ values, with exception for PFOS in one of the Swedish wells ($K_d$=1.5). Lab $K_d$ values were in all cases somewhat lower than the field $K_d$. Laboratory derived values for PFHpA, PFHxS, PFHxA and PFOA were <0.05 for aquifer materials from both sites, while PFOS and 6:2-FTS had slightly higher values with PFOS averaging 0.064 in the Swedish aquifer and 0.47 in the Spanish aquifer and 6:2-FTS averaging 0.24 in the Spanish aquifer.

Discussion: and conclusion: Low $K_d$ values and rapid desorption kinetics suggests that only low levels of PFAS will be retained by the aquifer during pumping. It also highlights the importance of managing contaminated groundwater at polluted sites since the results show that even long-chain PFAS such as PFOS and 6:2 FTSA would be present mainly in solution and therefore highly mobile in the groundwater flow.
TUE-AM-B5  Sustainable innovative drinking water treatment solutions for PFAS for large-scale water supply and reuse

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Introduction: Per- and polyfluoroalkyl substances (PFAS) concentration limits in drinking water have decreased or are going to be decrease worldwide. Drinking water is the single largest input to the human body, with the need of 2-2.5 L/day, e.g. for adults. However, current drinking water treatment techniques are inefficient considering the removal of PFAS from drinking water. Therefore, there is an urgent need to develop sustainable and innovative drinking water treatment solutions for large-scale water supply and reuse. The SIDWater project aims to ensure the sustainability of municipal drinking water supplies by developing new innovative treatment processes for removing PFAS and to provide a better alternative to the 'treat and release' of contaminants back into the environment, e.g. receiving water bodies.

Materials and Methods: The SIDWater project will investigate four case studies with different water sources in close collaboration with Swedish drinking water suppliers. SIDWater will assess a treatment train for drinking water supply and reuse including nanofiltration (NF), reverse osmosis (RO) membranes, foam fractionation, electrochemical oxidation, and (bio)filter solutions for a wide range of PFAS compounds.

Results: Our previous studies show that membrane processes, i.e. NF, RO, can separate PFAS by >98% generating a close by PFAS-free permeate that can be used for drinking water. Simultaneously, a concentrated PFAS rententate stream is produced which is handled by further concentrating using foam fractionation and electrochemical destruction. Foam fractionation utilizes air bubbles to PFAS rich rententate to take advantage of PFAS’s surfactant properties, thereby creating PFAS-rich foam, thereby reducing the captured PFAS volume to <1% of the feed volume and remove PFAS to >90% efficiency. This results in a relatively small PFAS volume for PFAS destruction using electrochemical oxidation.

Discussion and Conclusion: The innovative treatment train based on membrane processes to remove PFAS are more efficient compared to single treatment techniques (e.g. granulated activated carbon (GAC)) to meet higher stringent drinking water regulations. To close the PFAS loop and provide a sustainable process, PFAS can be destroyed in the foam fractionation concentrated solutions using electrochemical oxidation. Future work in the project will quantitatively examine the sustainability of this process.

Acknowledgments: The study was financially supported by FORMAS, grant number 2022-02108.
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1. Introduction:
Halogenated persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCB) remain a present issue in terrestrial soil systems despite accordant legacy bans⁵. Toxicity of these compounds and their effects on humans and the environment drives a need for remediation; however current techniques for removal and destruction remain cost prohibitive due to energy intensive processes and the use of large volumes of solvent⁶. POPs removal of such compounds via soil washing (SW) in conjunction with solvent reconditioning through UV-photocatalyzed chemical degradation shows promise for creating efficient and cost-effective treatment for the removal of POPs.

This paper will discuss the capabilities of a unique soil-washing additive desorption system (ADS) in conjunction with a purpose-built UV-light destruction technology for the extraction and elimination of Aroclor contamination from a field soil sample. This research is intended to explore more efficient soil-washing techniques through solvent capture and recycling.

2. Materials and Methods:
A hazardous waste site located in Guam with previously measured Aroclor contaminated soil was chosen for this investigation. Soil aliquots were taken from the field using a decision unit multi-increment sampling (DU-MIS) protocol⁶ to promote analyte homogeneity. Upon delivery, soil was spread into new aluminum baking trays to a max height of 15 cm and oven dried at 121°C for two hours in a Fisher Scientific Heratherm mechanical convection oven. The dry soil was then transferred into a W.S. Tyler RO-TAP sieve shaker and separated with a No.10 (2mm pore size) mesh stainless steel sieve. Following 5 minutes in the RO-TAP, above and below 2 mm grain size fractions were collected and stored in separate HDPE containers. The DU-MIS sampling procedure was completed on the less than 2 mm particle size fraction, and the resulting pre-treatment DU-MIS 'baseline' sample mass was stored in borosilicate glass sample containers at 4°C until analysis. The remaining bulk fraction of less than 2 mm material was transferred into a HDPE bucket and stored at ambient laboratory temperature until exposure to the ADS process.

To begin the ADS process, 200-gram soil aliquots were measured in glass containers from the bulk 2 mm fraction. Triplicate aliquots were prepared for each ADS experiment, and three independent experiments comprising of four, six, and ten ADS 'cycles' were completed. Each ADS 'cycle' consisted of mixing soil media with 200 mL of denatured alcohol blend for 15 minutes, separating the solvent and soil slurry via vacuum filtration, and finishing with passing 200 mL of fresh solvent through the soil bed while still in the vacuum filtration apparatus. Phase separation via vacuum filtration was completed in a borosilicate glass side arm flask and ceramic buncer funnel fitted with a 0.45-micron #4 Wattman filter. At the end of each cycle, soil samples were collected and stored at 4°C until analysis. Solvent samples from each experiment were collected and stored independently until all material was combined into one composite sample measuring 22.5 liters. This effluent from the ADS SW process was now the influent material to the UV degradation procedure.

The UV light reactor utilized a batch-flow design. Three time-based exposure experiments of one, four, and eight hours were conducted. Each experiment was conducted in duplicate. The reactor body and all parts were thoroughly cleaned with a soap water solution followed by a rinse with fresh, uncontaminated solvent at the beginning of testing and between all experimentation. To begin a test, 3L of contaminated solvent was added to the reactor chamber, the reactor lid was closed and sealed, and the pump was primed and allowed to come to a visual steady state flow. After steady state flow was reached, the UV-light system and timer were initiated simultaneously. At the designated timepoint for each experiment, the UV light system was cut off and sample was collected via an inline sampling port into a borosilicate glass PTFE lined sample container. Two additional experiments were conducted where solvent was loaded into the reactor, the pump was initiated, and lights were not engaged. These experimental runs served as light-off controls to verify the UV derived results.

In total, twelve soil samples and twelve solvent samples (see Table 1) were collected throughout all procedures. SGS North America Inc. Laboratories based in Orlando Florida conducted analysis on all samples. Preparation and analysis methods were completed using SW846 8082A⁴ and SW846 3562⁵.
### 3. Results:

**Table 1: 3rd Party Samples Collected**

<table>
<thead>
<tr>
<th>3rd Party ID</th>
<th>ADS Soil Experimental ID</th>
<th>3rd Party ID</th>
<th>Solvent Effluent Experimental ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>4cA</td>
<td>E1</td>
<td>1h Run A</td>
</tr>
<tr>
<td>S2</td>
<td>4cB</td>
<td>E2</td>
<td>1h Run B</td>
</tr>
<tr>
<td>S3</td>
<td>4cC</td>
<td>E3</td>
<td>4h Run A</td>
</tr>
<tr>
<td>S4</td>
<td>6cA</td>
<td>E4</td>
<td>4h Run B</td>
</tr>
<tr>
<td>S5</td>
<td>6cB</td>
<td>E5</td>
<td>8h Run A</td>
</tr>
<tr>
<td>S6</td>
<td>6cC</td>
<td>E6</td>
<td>8h Run B</td>
</tr>
<tr>
<td>S7</td>
<td>10cA</td>
<td>E7</td>
<td>Baseline A</td>
</tr>
<tr>
<td>S8</td>
<td>10cB</td>
<td>E8</td>
<td>Baseline A</td>
</tr>
<tr>
<td>S9</td>
<td>10cC</td>
<td>E9</td>
<td>Baseline B</td>
</tr>
<tr>
<td>S10</td>
<td>Baseline A</td>
<td>E10</td>
<td>Baseline B</td>
</tr>
<tr>
<td>S11</td>
<td>Baseline B</td>
<td>E11</td>
<td>4H Control Run A</td>
</tr>
<tr>
<td>S12</td>
<td>Baseline C</td>
<td>E12</td>
<td>4H Control Run B</td>
</tr>
</tbody>
</table>

![Figure 1: Aroclor Concentration in Post-ADS Soils](image)

Post-ADS treatment data for measured Aroclors as well as total Aroclor is presented above. Total Aroclor concentration was reduced from 21.22 PPM ('baseline') to 1.01 PPM after 10 cycles of ADS, a 95% decrease.
Figure 2: Aroclor Concentration over Time in UV Destruction System

Photocatalyzed UV destruction system data for Aroclor 1248, Aroclor 1254, and total Aroclor is presented above. The total Aroclor concentration was reduced to below the combined analytical limits of detection – 0.16 PPM (third party) – after 4 hours of UV exposure. Control run samples pulled at four hours showed no change from influent concentration.

Table 2: ADS Influent Use / Effluent Capture

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Total Ethanol Required (l)</th>
<th>Total Ethanol Captured (l)</th>
<th>% Ethanol Captured</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 Cycle</td>
<td>4.80</td>
<td>4.56 ± 0.05</td>
<td>95%</td>
</tr>
<tr>
<td>6 Cycle</td>
<td>7.20</td>
<td>6.75 ± 0.06</td>
<td>94%</td>
</tr>
<tr>
<td>10 Cycle</td>
<td>12.00</td>
<td>11.08 ± 0.30</td>
<td>92%</td>
</tr>
</tbody>
</table>

Efficient recovery was observed: on average, 94% of ADS solvent was recovered. Negligible loss of solvent was observed during use of the photocatalytic UV destruction system.

4. Discussion:
Upon conducting a comparison of presented research findings with existing literature, several points of agreement and disagreement were identified. A variety of SW parameters have been investigated previously such as leeching agent, concentration steps, solid to liquid ratio, and exposure time. Previous studies found that optimizing operational parameters, such as the nature of the extracting agent (EA), its concentration, and other operating conditions, is essential for enhancing extraction effectiveness (minimizing the use of EAs). In the current study, soil washing parameters remained the same aside from increasing the total amount of exposure cycles between experiments. The removal rates observed in this study align with published findings for SW with alcohol-based solvents in the range of 90-98%, but deviate from others with removal rates as low as 70%. It is important to consider the influence of soil physiochemical characteristics and the type of contaminant, as well as their interaction with the composition of the soil. The unique SW parameters employed consistently achieved the reduction of Aroclor concentration in soil below the analytical detection limits in all experimental trials, including the lowest exposure cycle experiment.
Even highly efficient soil washing procedures generate large amounts of contaminated solvent that must be stored or destroyed, increasing environmental impacts and limiting the financial viability of single-use solvents systems. This study observed efficient recovery of solvents, with recovery percentages consistently exceeding 90% in all experimental trials, demonstrating the feasibility of solvent capture for large scale applications. Additionally, the negligible solvent loss observed in the photocatalyzed UV destruction system suggests the potential for solvent reuse in subsequent remediation processes.

Removal of POPs from water or solvent systems may be achieved through either capture or destruction. Sequestration via granular activated carbon (GAC) requires constant maintenance and storage costs but has shown excellent removal of Aroclor contamination. Incinerators and high efficiency boilers, currently accepted by the US-EPA as approved POPs destruction methodology, generate large volumes of greenhouse gases and potentially harmful byproducts. Low-energy UV light driven destruction technologies have been designed as an alternative to sequestration/incineration and investigated for efficiency. SGS laboratory analysis of experimental samples reports non-detection for both Aroclor 1248 and 1254 in SW effluent after only four hours of UV exposure in the photocatalytic reactor. This implies the destruction of all Aroclor in solution to below the analytical limit of detection. These results are in alignment with research presenting on the complete degradation of Aroclor contamination via photolysis.

5. Conclusions:
Recycling solvents in soil washing processes offers a multitude of potential advantages, including cost reduction, enhanced sustainability, resource efficiency, simplified logistics, and improved system performance. As a result, it is a highly promising approach in the field of soil remediation. The solvent recovery of the ADS system tested averaged a 94% capture rate, and the solvent loss in the photocatalyzed UV destruction system was negligible. Post-treatment of the Aroclor-contaminated soil using the UV destruction system resulted in a total Aroclor concentration below the limits of detection. These outcomes underscore the viability of reusing the recovered solvent, thereby promoting cost reduction, enhanced sustainability, and minimized ecological impact within the system. ecoSPEARS will continue conducting further investigations to thoroughly explore and enhance the solvent reuse capabilities of the ADS UV-light destruction system.

ecoSPEARS is currently evaluating the ADS UV-light destruction system’s capacity for addressing Dioxins. Given the structural similarities between PCBs and dioxins, it is anticipated that the ADS-photocatalyzed UV destruction system will yield similar outcomes as the Aroclor studies. To date, the ADS system has exhibited success in extracting Dioxins from soil, with the subsequent destruction process being the next area of assessment. Current industry research has found high success in the removal of dioxins using soil washing, but consistently generated volatile organic compounds (VOC) during the process. In contrast, both the ADS soil washing system and the photocatalyzed UV destruction system exhibit non-combustible and non-thermal properties and hold promise for minimizing or preventing VOC generation during dioxin destruction.

Through ongoing research and testing, ecoSPEARS aims to develop efficient and sustainable soil-washing techniques with solvent capture and recycling in the ADS-Photocatalyzed UV destruction system for various Persistent Organic Pollutants.

6. References:
TUE-AM-B6  Soil-Washing Technologies in Conjunction with UV-catalyzed Photodegradation of Aroclor Contamination: A Solvent Capture Approach


TUE-AM-C1  Characterizing and risk assessment of human exposure to Organophosphorus Flame Retardants: Merging exposure markers of multiple pathway and biomonitoring data

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Introduction: Organophosphate flame retardants (OPFRs) are extensively used as alternatives to brominated flame retardants and widely applied in commercial products. Human exposure to OPFRs has been raised the great concern due to associations with potential health effects, especially in susceptible populations. The present study aimed to explore the exposome of OPFRs for children and their paired family caregivers in the indoor and outdoor environment.

Materials and Methods: The indoor and outdoor air, house dust, skin wipe, and paired urine samples of residents in 89 households were collected. 11 OPFRs in indoor and outdoor air, house dust, skin wipe samples were analyzed with gas chromatography/mass spectrometry. In addition, 5 OPFRs urinary metabolites (bis-butoxyethyl phosphate (BBOEP), bis (1,3-dichloro-2-propyl) phosphate [BDCIPP], bis-(1-chloro-2-propyl) phosphate [BCIPP], dibutyl phosphate [DBP] and diphenyl phosphate [DPHP]) were analyzed using a liquid chromatography-tandem mass spectrometry. The spearman correlation was used to examine the correlations between the OPFRs in the paired samples. Principal component analysis was applied to determine the dominant OPFRs in the paired samples.

Results: TBOEP was the most abundant OPFRs followed by TCIPP in house dust and skin wipe. TCIPP was responsible for almost all OPFRs content in indoor and outdoor air from households. The urinary concentrations of adults and children ranged from 0.176 to 0.475 µg/L and 0.192 to 0.683 µg/L, respectively. Total OPFRs dose of dermal absorption was higher than the other three exposure pathways. The average daily exposure dose of TBP back estimated from urinary DBP concentrations was significantly positively associated with dermal exposure to TBP. The established multiple exposure-biomonitoring model (MEBM) showed that most of the internal dose in caregivers and children was contributed from multiple exposure pathways via dermal absorption, inhalation of indoor air, inhalation of outdoor air, and ingestion of house dust. The non-carcinogenic and carcinogenic risk assessment showed that the exposure risk is acceptable and cause no significant damage.

Discussion and Conclusions: Dermal absorption is the main exposure pathway of OPFRs but inhalation of indoor/outdoor air and ingestion of house dust cannot be ignored. The significant correlation was demonstrated between the internal dose and external dose of OPFRs via inhalation of indoor/outdoor air and dermal absorption.

Acknowledgments: We thank the participants for their contributions, and our colleagues at the Research Center of Environmental Trace Toxic Substances for their sampling support. This work was supported by grants from NSTC 107-2314-B-006 and 108-2314-B-006 -038 -MY2 from the National Science and Technology Council, Taiwan.
TUE-AM-C2 Current background contamination levels of POPs in the general Walloon population (Belgium)

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Introduction:
The assessment of exposure levels of the general population to Persistent Organic Pollutants (POPs) was of high scientific concerns since the 2000s with the implementation of the Stockholm Convention and the progressive inclusion of new chemicals in this blacklist. This last decade, with the contamination level decrease and the emergence of less persistent alternatives, the attention of part of the scientific community has shifted, and the number of biomonitoring studies focused on legacy POPs such like PCBs, Organochlorine (OC) pesticides, or brominated flame retardants has declined. Moreover in Belgium, while biomonitoring surveys have been implemented in Flanders for more than 20 years, human exposure in the Walloon Region to POPs remained poorly documented. To fill this gap, the BMH-Wal project was launched in 2019 to provide background contamination levels for the Walloon population for several inorganic and organic pollutants, including various classes POPs such like PCBs, OC pesticides, polybrominated diphenylethers (PBDEs), and perfluoroalkyl substances (PFAS).

Materials and Methods:
Study population: In 2019-2020, 283 adolescents (12-19 years old) were recruited through schools representatively spread over Wallonia, while 261 adults (20-39 years old) were enrolled through Public Institutions.

Chemical analyses: Perfluorohexanesulfonic acid (PFHxS), linear perfluorooctane sulfonic acid (PFOS), perfluorohexanesulfonic acid (PFHxS), perfluorohexanoic acid (PFHxA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA) were analyzed by off-line solid phase extraction (SPE) followed by liquid chromatography coupled to tandem mass spectrometry1. PBDE-28, PBDE-47, PBDE-99, PBDE-100, PBDE-153, PBDE-154 and PBDE-183 were determined using a previously published analytical method2, while PCB-118, -138, -153, -180, and 14 OC pesticides or metabolites including among other hexachlorohexane isomers (HCH), hexachlorobenzene (HCB), chlordane and oxychlordane, dieldrin, nonachlor, β-endosulfan, and 4,4’- dichlorodiphényldichloroéthylène (4,4’-DDE) were extracted by liquid-liquid extraction, purified by SPE, and injected on a GC-MS/MS operating in negative chemical ionization.

Results:
Among the 14 OC pesticides, only HCB, b-HCH and 4,4’-DDE were quantified in respectively 19%, 3% and 7% of the samples. PCB-153 and -180 were the most abundant congeners with mean levels of 18.6 and 13.9 ng/g lip. Regarding PBDEs, only -47 and -153 were detected in 29% and 22% respectively, with concentrations up to 4.1 ng/g lip and 4.9 ng/g lip. PFOA and PFOS were the most abundant PFAS measured with median concentrations of 1.1 and 1.8 µg/L, while PFHxA and PFHpA were never or sporadically measured.

Discussion and Conclusion:
As expected, significant higher levels were measured in adults compared to adolescents for PCBs and OC pesticides, but also for some PFAS (PFHxA, PFHxS and PFOS) and for PBDE-47 and PBDE-153. Women showed statistically lower levels than men for some PFAS (PFOA, PFNA and PFHxS), likely explained by the elimination of PFAs during menstruation or breastfeeding. On the other hand, no gender difference was observed for PCBs nor PBDEs. Serum PFAS concentrations in Walloons are approximately twice lower than those measured 5 years earlier in a Belgian population (from Liege)3, but compared to more recent studies, they were similar to those reported from Europe or North America. PBDEs serum concentrations observed in Wallonia were very low, but comparable to those from Europe, and far lower than in United States or Canada. Finally, levels of PCB-153 and -180 were between 2 and 3 fold lower than those reported in Belgium in 2015. In conclusion, this study confirms the gradual decline in exposure to POPs in the European population over the past 10 years.

References:
1. Introduction:
Building on the two rounds of exposure studies with human milk coordinated by the World Health Organization (WHO) in the mid-1980s and 1990s on polychlorinated biphenyls (PCB), polychlorinated dibenzo-p-dioxins and furans (PCDD and PCDF), five expanded studies on persistent organic pollutants (POPs) were performed between 2000 and 2019. After the adoption of the Stockholm Convention on POPs in 2001, WHO and the United Nations Environment Programme (UNEP) collaborated in joint studies starting in 2004. The collaboration aimed at provision of POPs data for human milk as a core matrix under the Global Monitoring Plan (GMP) to assess the effectiveness of the Convention as required under Article 16. Over time, the number of analytes in the studies expanded from the initial 12 POPs targeted by the Convention for elimination or reduction to the 30 POPs covered under the Convention and two other POPs proposed for listing as of 2019. In the studies between 2000 and 2019, 82 countries from all five United Nations regions participated, of which 50 countries participated in more than one study. For the human milk samples of the 2016-2019 period, results are available for the full set of 32 POPs of interest for the Convention until 2019 (UNEP, 2020): (i) the 26 POPs listed by the start of the study in 2016, (ii) 2,2′,3,3′,4,4′,5,5′-decabromodiphenyl ether (BDE 209) and short-chain chlorinated paraffins (SCCP) listed in 2017, (iii) dicofol and perfluorooctanoic acid (PFOA) listed in 2019, (iv) medium-chain chlorinated paraffins (MCCP) and perfluorohexane sulfonic acid (PFHxS) proposed to be listed. This is a unique characteristic among the core matrices under the GMP. The data are publicly available in the Data Warehouse of the Stockholm Convention Global Monitoring Plan (GMP DWH) (GMP DWH, 2020).

The presentation of the concept, analysis, results and discussion of these global studies for the 32 POPs is a complex task which cannot be fulfilled in one publication. Therefore, a series of publications was prepared for the compendium “Persistent organic pollutants in human milk” (Malisch, Fürst and Šebková, 2023). This book presents and discusses the results for five groups of chlorinated and brominated compounds: (i) PCB, PCDD and PCDF, (ii) chlorinated pesticides and industrial chemicals; (iii) polybrominated substances; (iv) chlorinated paraffins; and (v) polychlorinated naphthalenes. Separate chapters assess the time trends derived from countries with repeated participation for (i) PCB, PCDD and PCDF, (ii) selected chlorinated pesticides and (iii) the listed perfluoroalkyl substances. Furthermore, a chapter describes a risk-benefit analysis for the breastfed infant regarding dioxin-like compounds.

The present paper summarizes the overall conclusions and key messages of the WHO- and UNEP-coordinated exposure studies.
2. Materials and Methods:
In all rounds, the design was based on collection of a number of individual samples and preparation of pooled samples following a standardized protocol that was supervised by national coordinators. Equal aliquots of individual samples were combined to give composite samples, which are considered representative of the average levels of the analytes of interest in human milk for a certain country or subgroup/region of a country at the time of sampling. The study design, analytical methods, and quality control data for determination of chlorinated and brominated compounds at the WHO- and UNEP-coordinated human milk studies 2000–2019 are described in the compendium.

3. Results, Discussion, and Conclusions:
3.1 Efficient and effective tool with global coverage as key contributor to the GMP
These studies are an efficient and effective tool with global coverage that serve as the key contributor to the GMP. Following collection of a large number of individual samples (usually 50) fulfilling protocol criteria, pooled samples are prepared using equal aliquots of individual samples (physical averaging) and are considered to be representative for a country, subgroup or subpopulation at the time of the sampling. The analysis of representative pooled human milk samples by dedicated Reference Laboratories meeting rigorous quality criteria contributes to reliability and comparability and reduces uncertainty of the analytical results. Additionally, this concept is very cost-effective and environmentally friendly.

3.2 Regional differentiation
These studies can be used for regional differentiation based on concentrations of various POPs between and within the five UN Regional Groups (African Group, Asia-Pacific Group, Eastern European Group, Group of Latin American and Caribbean Countries and Western European and Others Group). For some POPs, a wide range of contamination with up to three orders of magnitude among concentrations was found, even for countries in the same UN region. Some countries had levels within the usual range for most POPs, but high concentrations for certain POPs. Findings of concentrations in the upper third of the frequency distribution may motivate targeted follow-up studies rather than if the observed level of a POP is found in the lower third of frequency distribution. However, the level of a POP has to be seen in the context of the sampling period and the history and pattern of use of the POP in each country. Therefore, results are not intended for ranking of individual countries but rather to distinguish broader patterns.

3.3 Assessment of time trends
These studies can provide an assessment of time trends, as possible sources of variation were minimized by the survey concepts building on two factors (sampling design, analysis of the pooled samples by dedicated Reference Laboratories). For a first general estimation of time trends, the median or mean concentrations in UN Regional Groups can be compared over the five surveys in five equal four-year periods between 2000 and 2019. However, the variation of the number of countries participating in a UN Regional Group in a certain period can influence the median or mean concentrations. A more precise approach is the assessment of temporal trends considering only results of countries with repeated participation in these studies.

The reduction rates in countries should be seen in context with the concentration range: A differentiation between high levels and those in the range of background contamination is meaningful. If high levels are found, sources might be detected which could be eliminated. This can lead to significant decrease rates over the following years. However, if low background levels are reported, no specific sources can be detected. Other factors for exposure, e.g. the contamination of feed and food occurring by air via long-range transport, cannot be influenced locally.

For most countries, however, only very few time points are available for most POPs of interest, which prevents the derivation of statistically significant temporal trends in these cases. Yet, the limited existing data can indicate decreasing or increasing tendencies in POP concentrations in these countries. Furthermore, pooling of data in regions allows the derivation of statistically significant time trends in UN Regional Groups and globally. Overall time trends using the data from countries with repeated participation were calculated by the Theil-Sen method. Regarding the median levels of the five UN Regional Groups, a decrease per 10 years by 58% was found for dichlorodiphenyltrichloroethane (DDT; expressed as “DDT complex”: sum of all detected analytes, calculated as DDT), by 84% for beta-hexachlorocyclohexane (beta-HCH), by 57% for hexachlorobenzene (HCB), by 32% for polybrominated diphenyl ethers (PBDE), by 48% for perfluorooctane sulfonic acid (PFOS), by 70% for PCB and by 48% for PCDD and PCDF (expressed as toxic equivalents). In contrast, the concentrations of CP as an “emerging POP” showed increasing tendencies in some UN Regional Groups. On a global level, a statistically significant increase of total chlorinated paraffins (total CP including SCCP [listed in the Convention in 2017] and MCCP [proposed to be listed]) concentrations in human milk of 30% over 10 years was found.
3.4 Relative importance ("ranking") of the quantitative occurrence of POPs

The studies can provide the basis for discussion of the relative importance ("ranking") of the quantitative occurrence of POPs. This, however, requires a differentiation between two subgroups of lipophilic substances ([i] dioxin-like compounds, to be determined in the pg/g [=ng/kg] range, and [ii] non-dioxin-like chlorinated and brominated POPs, to be determined in the ng/g [=µg/kg] range and the more polar perfluorinated alkyl substances (PFAS), reported on a product basis [as pg/g fresh weight] or on volume basis [ng/L]). For this purpose, results for the complete set of the 32 POPs of interest for the 2016-2019 period were considered.

By far, the highest concentrations of lipophilic substances were found for DDT (expressed as "DDT complex"; maximum: 7100 ng/g lipid; median: 125 ng/g lipid) and for total CP; maximum: 700 ng total CP/g lipid; median: 116 ng total CP/g lipid). PCB was next in the ranking and had an average concentration about an order of magnitude lower than the average of the total CP concentrations (Figure 1).

![Figure 1: Range of concentrations of lipophilic chlorinated and brominated POPs in human milk (ng/g lipid) from 43 countries in the period 2016-2019 (median with error bars indicating the minimum and maximum)](image)

The high CP concentrations were predominantly due to MCCP. If the pooled samples from mothers without any known major contamination source nearby showed a high level of CP, some individual samples (e.g., from local populations close to emission sources, as a result of exposure to consumer products or from the domestic environment) might even have significantly higher levels. Due to the mother’s dietary and environmental exposure, the lactational intake of SCCP and MCCP by the breast-fed infant is on the microgram level and may motivate targeted follow-up studies and further measures to reduce exposure (including in the case of MCCP, regulatory efforts, e.g. the restriction of use in products).
3.5. Conclusions:
The seven rounds of WHO- and UNEP-coordinated human milk exposure studies are the largest global survey on human
tissues with a harmonized protocol spanning over the longest time period and carried out in a uniform format, including the
determination of the POPs of interest in pooled samples representative for countries or subgroups by designated reference
laboratories. Thus, these rounds are an effective tool to obtain reliable and comparable data sets of POPs in this core matrix
of the GMP. A comprehensive set of data covering all POPs targeted by the Stockholm Convention, in all UN Regional Groups
and covering a span of almost 20 years allows the evaluation of this human milk data from various perspectives. A three-
dimensional view is performed using the three pillars for assessment of this comprehensive data set, namely: analytes of
interest, regional aspects, and time trends. This data can identify possible problems for future targeted studies and interventions
at the country, regional and global levels. Long-term trends give an indication of the effectiveness of measures to eliminate
or reduce specific POPs, with decreasing levels for POPs found at elevated levels in earlier rounds, but increasing tendencies
for chlorinated paraffins. The continuation of these exposure studies is important for securing sufficient data for reliable time
trend assessments in the future. Therefore, it is highly recommended to continue this monitoring effort, particularly for POPs
that are of public health concern.

4. Acknowledgments:
We acknowledge the contribution and support from WHO and UNEP, the national coordinators of the jointly coordinated
exposure studies, assisted by the respective health, laboratory, and administrative staff – and last, but not least, all mothers
providing human milk.

The special issue (compendium) with publication on open accessible channels and in printed forms was developed based on
a Project Cooperation Agreement between UNEP and CVUA Freiburg in the framework of the projects titled “Implementation
of the POPs Monitoring Plan in the Asian Region under the Stockholm Convention” and “Continuing Regional Support for the
POPs Global Monitoring Plan under the Stockholm Convention” in the Africa, Pacific and Latin-American and Caribbean Region,
funded by the Global Environment Facility (GEF) and in close collaboration with and support of CVUA Freiburg.

5. References:
1. GMP DWH, 2020 Hůlek, R., Borůvková, J., Kalina, J., Bednářová, Z., Šebková, K., Hruban, T., Novotný, V., Ismael, M., Klánová
platform and on-line tool for the analysis of global levels of POPs in air, water, breast milk and blood [online]. Masaryk
publication; all articles open access)
tabid/2509/Default.aspx
TUE-AM-C4 Assessment of e-waste occupational exposure to PCBs and PBDEs using settled dust and silicone wristbands analysis

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Introduction:
Due to their industrial origin and constituents, electric and electronic equipment may contain organic contaminants, such as polychlorobiphenyls (PCBs) and polybromodiphenylethers (PBDEs). Workers involved in the e-waste recycling are thus potentially exposed to these toxic chemicals via dust inhalation/ingestion and/or dermal contact. The present work is part of the Human Biomonitoring Initiative (HBM4EU) e-waste study [1]. Its objective was to investigate the occupational exposure to PCBs and PBDEs in e-waste workers by (i) analyzing hygiene samples such as floor settled dust (FSD) collected in e-waste premises and personal silicone wristbands (SWB) worn by workers and controls, (ii) identifying potential differences of exposure depending on the type of e-waste processed and (iii) evaluate the correlation and relevance of results obtained from both matrices.

Materials and Methods:
According to the HBM4EU e-waste study protocol previously published [1], FSD samples were collected with a vacuum cleaner in HEPA filter bags in e-waste processing facilities. In parallel, pre-conditioned SWB were worn by exposed workers and controls, during working hours only and for one complete workweek. After reception to the lab, FSD samples were sieved (<63 µm) and submitted to accelerated solvent extraction with n-hexane/acetone. The obtained extracts were then analyzed by GC-EI-MS in SIM mode. Alongside this, SWB were cut and extracted twice by 2h rotative shaking with ethyl acetate. An aliquot of the extract was cleaned-up with two successive SPE steps (Oasis HLB and Sep-Pak Silica) and then analyzed by GC-NCI-MS with SIM mode acquisition. For both methods, appropriate calibration curves and QCs were prepared for each batch of samples to ensure reliable quantification of five PCBs (#101, #118, #138, #153, #180) and eight PBDEs (#28, #47, #99, #100, #153, #154, #183, #209). The limits of quantification (LOQ) were set at 0.1 µg/g (1 µg/g for BDE209) for FSD and 1 ng/WB (10 ng/WB for BDE209) for SWB.

Results:
The percentage of FSD samples above the LOQ ranged from 24 to 46% for PCBs and from 2 to 73% for PBDEs, with BDE99, BDE183 and BDE209 being the most frequently detected (63%, 66% and 73%, respectively). SWB presented a higher percentage of samples ≥ LOQ for both PCBs (51-83%) and PBDEs (19-94%) with BDE209 found in 94% of the samples. When grouping the results of FSD by sub-categories (i.e. type of e-waste processed: (i) batteries, (ii) white goods, (iii) brown goods, and (iv) metals and plastics), statistical analysis (Kruskal-Wallis H test followed by post-hoc Dunn test) revealed significant differences (p<0.05) for PCB180, BDE47, BDE99, BDE100, BDE153, BDE183 and BDE209 between the sub-groups. The same tests conducted with SWB levels highlighted significant differences between the five sub-categories (4 types of e-waste processed and controls) for all compounds of interest, except BDE28. Moreover, strong and positive Spearman correlations (r_s) between FSD and SWB results were observed for all compounds, except BDE28 (r_s: 0.16 for all PCBs, 0.37-0.79 for other PBDEs).

Discussion and Conclusion:
These findings suggest different PCB/PBDE exposure levels for workers depending on the type of e-waste processed. The strong and positive correlations observed between FSD and SWB results support the use of these two types of hygiene sampling approaches for reliable external exposure assessment and characterization. Further investigation on human biomonitoring samples (blood and hair) are currently under consideration to assess the internal dose and better evaluate the contribution of occupational exposure compared with other potential sources of contamination.

Acknowledgments:
Co-authors are grateful to HBM4EU e-waste study team and all experts involved in any way in the present work. Many thanks are also addressed to companies and workers for their participation and lab technicians for their analytical work.

References:
Introduction: Chlordane (CHL) has been used in Japanese wooden houses as an insecticide for white ants. While it was discontinued from production and use in Japan in 1986, CHL is still detected in biological samples, such as breast milk (Fujii et al., 2023). A possible route of human exposure to CHL is the ingestion of house dust, as CHL persists in the indoor environment for a long time. However, reports on CHL concentrations in house dust are still scarce. The primary goal of the present study was to investigate the occurrence of CHL in house dust and the associated daily intake through dust ingestion. To achieve this goal, we investigated vacuum cleaner dust concentrations of CHL and selected organic pollutants in Fukuoka, Japan.

Materials and Methods: Dust samples (n=27) were collected from homes within the Fukuoka Prefecture and neighbouring area between August and November 2017 according to previous methods (Liu et al., 2011). Dust was retrieved from the used bags of household vacuum cleaners at each premises, and placed into polyethylene bags for transport to the lab, where samples were sieved to a <500 µm fraction. Selected compounds such as CHL (oxychlordane (OxC), trans-nonachlor (TC), cis-chlordane (CC), trans-nonachlor (TN), cis-nonachlor (CN)), BDE 209, decabromodiphenyl ethane (DBDPE), and dechlorane plus (DP) were analyzed by gas chromatography and mass spectrometry in electron capture negative ionization mode. Estimated daily intake (EDI) of CHL was calculated assuming a dust intake of 50 mg/day and a body weight of 60 kg. The EDI was compared with the reference dose (RfD) of CHL (0.0005 mg/kg/day).

Results: Among the CHL, TC, CC, TN and CN were detected in 100% of samples and OxC was in 30%. The median $\Sigma$CHL concentration was 8.8 ng/g dust and levels ranged from 3.4 to 876 ng/g. The median concentrations among the compounds were highest for TC (3.8 ng/g, maximum 316 ng/g), followed by CC and TN (2.1 ng/g, maximum 230 ng/g for CC, 207 ng/g for CN, respectively), then CN (0.87 ng/g, maximum 128 ng/g). The median concentrations among the other groups of compounds were highest for DBDPE (446 ng/g, maximum 6608 ng/g), followed by BDE 209 (277 ng/g, maximum 16220 ng/g), $\Sigma$DPs (2.8 ng/g, maximum 27 ng/g). EDI for $\Sigma$CHL was 0.0073 ng/kg/day, which is 1.5×10^{-5} times lower than the RfD.

Discussion and Conclusion: More than 30 years after the ban, CHL was still detected in house dust in Japan. Although the levels are three orders of magnitude lower than BDE 209 and DBDPE, residents of certain dwellings are expected to have higher levels in their blood and breast milk due to long-term exposure to CHL. Further investigations are warranted.

References:
Human Exposure

G. Schoeters & P. Brambilla

TUE-AM-C6  Health Assessment Of Emerging Persistent Organic Pollutants (POPs) In Pm2.5 From Municipal Solid Waste Incinerators In Northern Taiwan

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2 Graduate Institute of Environmental Engineering, National Central University.

Introduction:
In recent decades, PM2.5 has emerged as a significant global health concern (Song et al., 2022). It is mainly composed of metal elements, water-soluble ions, and organic compounds. Among these elements, Polychlorinated Dibenzo-p-dioxins and Dibenzo-furans (PCDD/Fs), Polychlorinated Biphenyls (PCBs) and Polychlorinated Naphthalenes (PCNs) have attracted many interests (Moon et al., 2012). Due to their lipophilic and hydrophobic properties, they are often found in soil (Wang et al., 2016), food (Guo et al., 2019), surface water (Han and Currell, 2017), as well as in polar regions and Tibet plateau (Shao et al., 2019). Previous studies have instructed that PCDD/Fs, PCBs and PCNs are inadvertently discharged into the environment from various industrial and thermal activities, including metallurgical procedures (Hu et al., 2014; Hung et al., 2012, 2015), municipal waste incinerator (Chi et al., 2005; Yang et al., 2019), vehicle exhaust (McKay, 2002), coking industry (Liu et al., 2009, 2010), combustions of coal and wood (Lee et al., 2005; Bai et al., 2017). Municipal waste has become a serious matter in Taiwan. Municipal solid waste incineration (MSWI) has become a controversial method of waste disposal due to release of persistent organic pollutants including PCDD/Fs, PCBs and PCNs respectively. Once these pollutants enter the body, they can have adverse effects on human health. Interestingly, most research discussed the characteristics, toxicity and sources of PCDD/Fs and PCBs, but there was limited evidence about PCNs. Therefore, this study investigates the seasonal variations of ambient PCNs in PM2.5 of northern Taiwan, and further analyses their possible pollution sources and assess their health risks.

Materials and Methods:
Taipei has been selected as the study area, being the most populous city with a population density of approximately 2.6 million (Chi et al., 2022), located in Northern Taiwan. Additionally, a high-volume sampler (HV-1000R and Digital) with a flow rate of 500 L-1 was equipped with quartz fiber filters and polyurethane foam (PUF), along with XAD2 to collect the solid and vapor phase PM2.5, over a 68 h periods. After sampling, the samples were extracted by Soxhlet extractor with a mixture of solvents (n-hexane and acetone; vol/vol, 1:1; for 8 hours; 100% Toluene for 16 hours). Then the extracts were cleaned-up with a multi-layer acid silica gel column and activated carbon to produce pure product. Moreover, the chemical analysis included 17 PCDD/Fs, 12 PCBs, and 20 PCNs, respectively. Besides, AERMOD was used to simulate ambient sampling stations at the elementary school in Shulin as MWI vicinity from January to December, 2021. Additionally, Positive Matrix Factorization (PMF) determines the source profiles (matrix F) and contributions (matrix G) to the observed data (matrix X), while minimizing the model residuals (matrix E). The technique was employed to identify the sources of air pollution in the selected location. Moreover, to calculate the concentration of POPs through various media such as water, leafy vegetables, root vegetables, grains, pork, beef, poultry, and fish the multimedia model was employed. Both exposure dose (ingestion, inhalation and dermal) and inhalation and non-inhalation cancer risk in different ages in different area were calculated.

Results and Discussion:
No seasonal changes in the levels of PCNs were observed during the entire period. In the last 20 years, the concentration of PCDD/Fs in TSP at Taipei city has seen a drastic decline from 132 to 15.8 fg TEQWHO/m3 during winters and from 59.3 to 18.6 fg TEQWHO/m3 during summers as given in Fig. 1. Additionally, the PCBs in PM2.5 were largest during winter (0.090-0.400 fg TEQWHO/m3) followed by spring (0.080-0.160 fg TEQWHO/m3), summer (0.010-0.050 fg TEQWHO/m3) and autumn (0.030-0.070 fg TEQWHO/m3). Also, SO₂⁻ (1.86 µg/m³), NO₃⁻ (0.35 µg/m³) and Na⁺ (0.10 µg/m³) were the primary components of PM2.5. Based on PMF analysis, PCDD/Fs were divided into 4 Factors including traffic emission (3.50%), long range-transport (9.07%), Sintering plant (77.9%) and municipal waste incinerator (MWI) (9.50%). PCBs were divided into 3 Factors as woodchip boiler (68.3%), MWI (21.0%) and EAF (10.8%). PCNs were divided into 3 Factors as MWI (64.9%), secondary copper smelter (13.0%) and copper sludge smelter (22.1%) as indicated in Fig. 1b and 1c. Moreover, the average concentrations of PCDD/F, PCBs, and PCNs in the soil at the study site were consisted of 0.958-7.53 fg TEQWHO/m3/day, 0.008-0.160 fg TEQWHO/m3/day, 0.013-1.62 fg TEQWHO/m3 day and 3.78-13.7 fg TEQWHO/ respectively as indicated in Table. 1. In addition, exposure to high doses of pollutants (PCBs, PCDD/Fs and PCNs) were observed in adults aged 19-70 in the study area (Table. 2). The cancer risk assessment of PCDD/Fs, PCBs and PCNs were showed in Table. 3 PCDD/Fs dominated >95% in total cancer risk.
The age of 19-70 year-old people had the highest risk ($9.26 \times 10^{-9} - 1.12 \times 10^{-7}$) in comparison to 0-12-year-old and 12-18-year-old people. Non-inhalation cancer risk in the age of 19-70-year-old people had the highest risk ($4.00 \times 10^{-6} - 3.00 \times 10^{-5}$). The lowest was 0-12-year-old people ($1.71 \times 10^{-6} - 3.80 \times 10^{-5}$).

Finally, it was discovered that there was a heightened level of cancer risk amongst individuals between the ages of 19-70 ($4.00 \times 10^{-6} - 3.00 \times 10^{-5}$), although this stayed within an acceptable range.

Acknowledgements:
The authors gratefully acknowledge the Taiwan Environmental Protection Administration (EPA-106-E3S4-02-02 and EPA-107-E3S4-02-01), the Ministry of Science and Technology of Taiwan (MOST 110-2111-M-A49A-501-and NSC 111-2111-M-A49-001-) for financial support.

Reference:

![Fig. 1 Atmospheric PCDD/Fs concentration in particle and gas phase in TSP during 20 years](image-url)
MORNING BREAKOUT SESSIONS

10:00 - 12:00

Human Exposure
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Fig. 2 Congener profile of (a) PCDD/Fs, (b) PCBs and (c) PCNs in PM$_{2.5}$ in 2021.

Fig. 3 The source apportionment via PMF of (a) PCDD/Fs, (b) PCBs and (c) PCNs in 2021.

Table 1 Concentration of POPs in different media in PCDD/Fs, PCBs and PCNs

<table>
<thead>
<tr>
<th>Media</th>
<th>PCDD/Fs (range mean)</th>
<th>PCBs (range mean)</th>
<th>PCNs (range mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atmosphere (fg TEQ$<em>{\text{WHO}}$ /m$</em>{3}$-day)</td>
<td>0.958-7.53 (4.05)</td>
<td>0.008-0.160 (0.060)</td>
<td>0.013-1.62 (0.358)</td>
</tr>
<tr>
<td>Desperation (pg TEQ$<em>{\text{WHO}}$ /m$</em>{2}$-day)</td>
<td>1.73-2.65 (2.04)</td>
<td>0.074-0.262 (0.143)</td>
<td>0.026-0.104 (0.053)</td>
</tr>
<tr>
<td>Soli (pg TEQ$_{\text{WHO}}$ /g)</td>
<td>155.29-238 (183)</td>
<td>6.69-23.5 (12.8)</td>
<td>2.30-9.37 (4.77)</td>
</tr>
<tr>
<td>Water (pg TEQ$_{\text{WHO}}$ /g)</td>
<td>0.08-0.130 (0.100)</td>
<td>0.004-0.013 (0.010)</td>
<td>0.0012-0.050 (0.005)</td>
</tr>
</tbody>
</table>
TUE-AM-C6  Health Assessment Of Emerging Persistent Organic Pollutants (POPs) In Pm2.5 From Municipal Solid Waste Incinerators In Northern Taiwan

Table. 2 The exposure dose in PCDD/Fs, PCBs and PCNs

<table>
<thead>
<tr>
<th>Age</th>
<th>Inhalation dose (mg TEQ_{WHO}/kg-day)</th>
<th>Ingestion dose</th>
<th>Dermal contact dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-12</td>
<td>2.41E-13-2.29E-12</td>
<td>3.47E-09-5.84E-09</td>
<td>7.88E-17-1.30E-16</td>
</tr>
<tr>
<td>12-18</td>
<td>9.51E-14-9.03E-13</td>
<td>2.37E-09-3.99E-09</td>
<td>1.62E-17-2.67E-17</td>
</tr>
<tr>
<td>19-70</td>
<td>8.38E-14-7.96E-13</td>
<td>2.65E-09-4.46E-09</td>
<td>1.62E-17-2.67E-17</td>
</tr>
</tbody>
</table>

Table. 3 The inhalation and non inhalation cancer risk in PCDD/Fs, PCBs and PCNs

<table>
<thead>
<tr>
<th>Age</th>
<th>PCDD/Fs</th>
<th>PCBs</th>
<th>PCNs</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-12</td>
<td>7.70E-9-1.87E-7</td>
<td>2.15E-16-1.87E-9</td>
<td>8.52E-11-6.81E-8</td>
<td>7.79E-9-2.56E-7</td>
</tr>
<tr>
<td>12-18</td>
<td>2.61E-9-2.49E-8</td>
<td>9.46E-17-2.49E-8</td>
<td>2.89E-11-8.15E-9</td>
<td>2.63E-9-3.31E-8</td>
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<tr>
<td>19-70</td>
<td>9.16E-9-8.27E-8</td>
<td>2.93E-16-8.27E-8</td>
<td>1.02E-10-2.89E-8</td>
<td>9.26E-9-1.12E-7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age</th>
<th>PCDD/Fs</th>
<th>PCBs</th>
<th>PCNs</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-12</td>
<td>1.68E-6-2.79E-5</td>
<td>5.51E-13-2.55E-12</td>
<td>2.24E-8-9.72E-6</td>
<td>1.71E-6-3.80E-5</td>
</tr>
<tr>
<td>12-18</td>
<td>1.05E-6-5.21E-6</td>
<td>3.12E-13-1.14E-12</td>
<td>1.32E-8-1.91E-6</td>
<td>1.06E-6-7.12E-6</td>
</tr>
<tr>
<td>19-70</td>
<td>3.95E-6-2.20E-5</td>
<td>1.27E-12-5.00E-12</td>
<td>5.15E-8-8.33E-6</td>
<td>4.00E-6-3.00E-5</td>
</tr>
</tbody>
</table>
Kerstin Krätschmer*, Wout Bergkamp
Wageningen Food Safety Research (WFSR), Wageningen University & Research, Akkermaalsbos 2, 6708 WB Wageningen, The Netherlands

**Introduction:** Chlorinated paraffins (CPs) are high production volume chemicals of growing public health concern because of their ubiquitous presence in food samples, their persistence, and their potential accumulation potential. Previous market basket studies in Europe have shown that especially vegetable fats and oils are among the food groups with the highest observed CP content [1,2]. In a comparison of consumer behaviour data between twelve European countries 2017, the Netherlands had the third-highest fat and oil consumption per capita after Finland and Romania [3], with around 23 g/day for an average adult [4]. In order to make a first assessment of the dietary CP exposure of the Dutch population, 55 vegetable oil and fat samples collected 2021-2022 from the Dutch market were analysed to determine their content of short chain (SCCP), medium chain (MCCP) and long chain CPs (LCCPs). To also account for non-mainstream products, ethnic and wholesale supermarkets were specifically included in the sampling campaign.

**Materials and Methods:** In brief, the oil and fat samples were spiked with 0.1 mL of 13C10 -1,5,5,6,6,10-hexachlorodecane and dissolved in 10 mL n-hexane. To remove lipids, 5 mL concentrated sulphuric acid was added directly and mixed gently before storage overnight to form separate layers. The upper layer was collected and repeated liquid/liquid extraction with n-hexane was performed. Following further clean-up on a deactivated silica column eluted with 60 mL n-hexane, the eluate was collected and evaporated to 1 mL after a solvent change to isooctane. Analysis was done using a LC-ESI-HRMS instrument (R=140.000 FWHM, m/z 120-1500, full scan) with deconvolution of the resulting CP patterns for quantification based on available mixed CP standards.

<table>
<thead>
<tr>
<th>Oil type</th>
<th>Results in ng/g product</th>
<th>sum of CPs</th>
<th>SCCPs</th>
<th>MCCPs</th>
<th>LCCPs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palm oil</td>
<td>6000</td>
<td>4840</td>
<td>910</td>
<td>250</td>
<td></td>
</tr>
<tr>
<td>Palm oil</td>
<td>5670</td>
<td>2550</td>
<td>3120</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Palm oil, zomi</td>
<td>4560</td>
<td>1840</td>
<td>2710</td>
<td>(7)</td>
<td></td>
</tr>
<tr>
<td>Sesame oil</td>
<td>4350</td>
<td>4300</td>
<td>44</td>
<td>(8)</td>
<td></td>
</tr>
<tr>
<td>Palm oil</td>
<td>1520</td>
<td>580</td>
<td>910</td>
<td>30</td>
<td></td>
</tr>
</tbody>
</table>

**Results:** Overall, the sum of CPs observed in the samples ranged <LOQ to 6000 ng/g product (mean: 610 ng/g). Of the five vegetable oil samples with the highest sum of CP content, four were palm oils or zomi palm oils (Table 1). Remarkably, much of the CP content derived equally from SCCPs and MCCPs while in the sesame oil mostly SCCPs were observed. The 16 analysed palm oils ranged 70-6000 ng/g product for sum of CPs (mean 1370 ng/g), with SCCP:MCCP ratios ranging 0.6-10.3 (mean 2.8). Most of these samples were either unrefined or only cold filtered and retained their characteristic red, cloudy appearance.

**Discussion and Conclusion:** In half of the vegetable oil and fat samples, elevated CP levels above 100 ng/g product were observed. While palm oil was not included in the published studies, other oil types are in agreement with literature data: rice oil and some extra virgin olive oils dominated the ranking due to elevated MCCP levels, while blended and refined oils and vegetable ghee fell below the 100 ng CP/g product threshold. Worst-case dietary CP exposure through (palm) oil is 2.1 µg/kg bw/day for adults, almost 4-fold higher than exposure through edible oil in the other studies. Excluding palm oils, the mean dietary exposure through Dutch edible oils is in good agreement with the other European studies. Considering the very high findings in unrefined palm oil samples, further investigation into (SC)CP sources in the production chain and also levels in palm oil refinement side streams such a palm fatty acid distillates (used for animal feed production) seems prudent.

**Acknowledgments:** The Dutch Ministry of Agriculture, Nature and Food Quality is gratefully acknowledged for providing financial support to the authors (WOT-02-001-015).

**References:**
Can Chlorinated Paraffins (CPs) be Removed by Vegetable Oil Refining? A Study on Refining Removal Efficiencies and Vegetable Oils.

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Introduction: Chlorinated Paraffins (CPs) are a class of man-made chemicals for many applications. As a result of their high production volumes, they are now pollutants that are ubiquitously distributed over the globe. Dietary exposure to CPs through food has raised health concerns. To follow the EFSA Opinion, more studies related to the levels of CPs in various food categories from Europe have been conducted [1]. Additionally, there are indications for a partial removal of CPs during refinement [2]. To obtain insight into the CP removal efficiency of the refining process, 13 paired crude and refined vegetable oils refined by a Dutch contract refining company were studied for the removal efficiency of SCCPs and MCCPs and selective removal of congeners. The data were compared to 39 vegetable oils collected from local supermarkets.

Materials and Methods: Eight CP mixture standards (SCCPs with chlorination degree 51.5%, 55.5% or 63% and MCCPs with chlorination degree 42%, 52% or 57% were obtained for quantification. For the sample preparation, each oil sample was weighed and spiked with 13C10-1,5,5,6,6,10-Hexachlorodecane and dissolved in n-hexane. Concentrated sulphuric acid were added and the samples was put aside overnight after ultrasonication. The upper layer was collected and a repeated extraction was performed. The homogenized extracts in the volumetric flasks were applied to a column. The purified extracts were analysed using UHPLC-Orbitrap-MS. A deconvolution method was used to calculate the concentrations of SCCPs and MCCPs, with additional qualitative information on relative homologue group patterns of these CPs.

Results: Refining of vegetable oil can remove both SCCPs and MCCPs from crude oils (average removal: 83% and 71%, Table 1), respectively. While in the CP homologue group patterns of crude oils, decanes and tetradecanes were dominant; tridecanes and tetradecanes were predominant in refined oils. For the chlorination degree, a reduction was detected primarily for penta-chlorinated CPs when comparing crude and refined oil samples. The levels of CPs in refined oils were comparable with observed results of oils form supermarkets, where MCCPs also predominated over SCCPs.

Table 1: Concentrations of SCCPs and MCCPs in measured vegetable oil samples. Amounts in ng/g lw.

<table>
<thead>
<tr>
<th>Sample</th>
<th>SCCPs</th>
<th>MCCPs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Min</td>
<td>Max</td>
</tr>
<tr>
<td>Crude oils (refining company)</td>
<td>&lt;8</td>
<td>167</td>
</tr>
<tr>
<td>Refined oils (refining company)</td>
<td>&lt;8</td>
<td>13</td>
</tr>
<tr>
<td>Vegetable oils (local supermarkets)</td>
<td>&lt;8</td>
<td>78</td>
</tr>
</tbody>
</table>

Discussion and Conclusion: The results indicate that CPs are partially removed by the refining process, which is consistent with the results of previous study [2]. The removal of SCCPs is more efficient than MCCPs; CPs with a relatively short carbon chain length and low chlorination degree with higher volatility, are more likely to be removed by the heating procedure during the processing [3]. MCCPs were also more prevalent (than SCCPs) in supermarket purchased vegetable oils. Further studies are needed to investigate the origins of CPs in crude oils and into refining techniques for removal of highly lipophilic CPs.

Acknowledgements: The China Scholarship Council (grant number: 201908610201) and the Dutch Ministry of Agriculture, Nature and Food Quality (KB-37-002-023) is gratefully acknowledged for providing financial support. We would like to thank W. Bergkamp for assistance with the sample preparations and sample injections; I. Rietjens, J. Louisse. and K. Beekman for their contribution in this study; the Special Refining Company (SRC) for providing the paired crude and refined vegetable oil samples.

References:
1. Introduction:
Textiles and synthetic fibres are produced in huge quantities every year. It is estimated that more than 100 million tonnes were produced in 2018 (all uses, not just for clothing) (Patti et al., 2020). There are several environmental risks associated with this production, such as high energy consumption, excessive transportation, the production of packaging, and the emission of pollutants (such as bisphenol A – BPA (Wang et al., 2019) or phthalates (Li et al., 2019)) into wastewater during washing (Kolarik & Morrison, 2022; Patti et al., 2020). This high production also leads to the need to recycle or down-cycle materials that could be a source of contaminants in the new products. In addition, clothing is in direct contact with human skin, which can lead to dermal exposure to chemical components, additives and contaminants (Freire et al., 2019; Wang et al., 2019).

This study focused on chlorinated paraffins (CPs), which are used in various industrial applications, not only as plasticisers and flame retardants in plastics, particularly polyvinyl chloride (PVC) but also as additives in paints and textiles and in metalworking fluids (UNEP, 2015). While the possible addition in textiles is acknowledged in the literature (Gluge et al., 2016; Vetter et al., 2022), no exact concentrations in finished clothing and fibres have been published (to the best of our knowledge). Their monitoring in consumer products should therefore be sought, as there are several human health concerns, including endocrine disruption and possible carcinogenicity of short-chain chlorinated paraffins (SCCPs; carbon chain length of C10-C13) (UNEP, 2015).

Another group of CPs, medium-chain chlorinated paraffins (MCCPs; carbon chain lengths of C14-C17), which are toxic to aquatic organisms and harmful to rodent offspring (UNEP, 2022), have been found at low contents (3.7 ng/g) in dish towels in Germany (Gallistl et al., 2017). In addition, SCCPs and MCCPs have been repeatedly reported in various polymeric consumer products, with concentrations varying from units of µg/g to wt%. CPs have been found particularly in toys, sports equipment, cable insulation, and flooring (Guida et al., 2022; McGrath et al., 2021). Due to abrasion, polymeric products can be a source of CPs in indoor dust (Cao et al., 2019). Similarly, (micro)fibre from textiles have been described as part of urban and indoor dust (Chen et al., 2022).

The objectives of this study were: (i) to determine the CP content in textiles and (ii) to investigate the effects of washing on the CP content in textiles. Samples of both natural and synthetic fibres were analysed. It was expected that the latter would be more contaminated as CPs are additives of synthetic fibres.

2. Materials and Methods:
Clothing samples (socks, n=14; and T-shirts, n=14) from seven retail shops (in Prague, Czech Republic) and eshops were purchased as part of a market survey in spring 2020. Samples were not worn and were stored in a dry place at room temperature (similar to shops and homes) before analysis. Each sample was analysed before and after washing in a washing machine (at 30°C for 1 hour with the addition of detergent – washing gel). In the washing experiment, small strips (~200 cm² of a T-shirt and ~50 cm² of a sock) from all samples were washed together. After washing, the fabric strips were dried overnight at room temperature under normal conditions.

The sample preparation procedure is as follows: the textile sample was cut into small pieces (1x1 cm) and the sample (1 g) was extracted with 10 ml of n-hexane:dichloromethane (1:1, v/v, assisted by sonication) for 1 h. The extract was collected and the extraction was repeated twice with the addition of 10 ml fresh n-hexane:dichloromethane (1:1, v/v) to the sample. The combined extracts (30 ml) were then concentrated using a rotary vacuum evaporator (RVE). CPs were then isolated from the co-extracted matrix using solid phase extraction (SPE). Silica gel (0.5 g) was used and the CPs were eluted with 6 ml n-hexane:dichloromethane (3:1, v/v). The eluate was then concentrated using RVE, dried under a light stream of nitrogen and then dissolved in 0.5 ml syringe standard (containing 5 ng/ml PCB 166 in isooctane). Matrix components (e.g. oligomers, additives, other impurities) that were not retained on the SPE column were mineralised by adding a few drops of concentrated sulphuric acid. The organic layer was then transferred to a crimp vial and injected into GC-HRMS.
3. Results:
In this study, CPs were determined in T-shirt samples (n=14) and sock samples (n=14) – see Figure 1. In the new, unwashed samples, SCCPs above LOQ (5 ng/g) were found in all cases, with concentrations ranging from 17.1 to 4040 ng/g (mean 933 ng/g, median 209 ng/g). MCCPs were found in 26 of 28 samples (93%) above LOQ (10 ng/g), with concentrations ranging from <10 to 2240 ng/g (mean 324 ng/g, median 148 ng/g).

The influence of washing was tested (see Figure 1). In the washed samples, the concentrations changed and CPs were found above the LOQs in all samples. SCCP contents ranged from 19.4 to 4180 ng/g (mean 598 ng/g, median 438 ng/g). MCCP contents were 14.7 – 1560 ng/g (mean 220 ng/g, median 147 ng/g). The individual samples behaved differently: (i) excessively emitting CPs; (ii) being contaminated; (iii) retaining the original levels of CPs.

It was expected that the content of CPs would be influenced by the material used. Table 1, therefore, groups the results according to the materials used. There were samples consisting entirely of cotton (T-shirts n=8; socks n=0) and samples with a significant proportion of synthetic fibres (T-shirts n=2; socks n=13). The rest of the samples did not contain any information about the composition.

The CP profiles also changed for some samples (both samples with a significant proportion of synthetic fibres and samples that consisted exclusively of cotton) – see Figure 2.

Table 1: The CP concentrations in the analysed samples, grouped by the material used. Contents in ng/g.

<table>
<thead>
<tr>
<th>Sample material</th>
<th>SCCPs</th>
<th>MCCPs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100% Cotton</td>
<td>100% Cotton</td>
</tr>
<tr>
<td></td>
<td>new (n=8)</td>
<td>washed (n=8)</td>
</tr>
<tr>
<td>Mean</td>
<td>70.6</td>
<td>95.4</td>
</tr>
<tr>
<td>Median</td>
<td>57.0</td>
<td>98.3</td>
</tr>
<tr>
<td>Min - Max</td>
<td>20.0 - 183</td>
<td>19.4 - 214</td>
</tr>
</tbody>
</table>

Figure 1: SCCP and MCCP concentrations in clothing (n=28) before and after washing in the washing machine with detergent.
4. Discussion:
Synthetic fibres were expected to be the source of CPs, as CPs can be used as additives to improve material properties. CPs were still detected in cotton textiles, but in much lower amounts (Table 1). Their sources could be additives in dyes, contamination of the source plants or the final fibres from production. Secondary contamination of the finished clothing cannot be ruled out either (through transport, packaging or dust deposition before sampling). The same applies to the synthetic fibres (non-intentional origin of CPs) (Saini et al., 2017).

On the other hand, the synthetic fibres analysed in our study contained lower levels (means and medians) of CPs than plastic materials (toys, food packaging, consumer products) recently reported in other papers (Guida et al., 2022; McGrath et al., 2021; Zhang et al., 2023).

Washing had a strong effect on CP levels in textiles (as seen in Table 1). The effect of washing on another group of organohalogen compounds – PCBs – in textiles was studied by Kolarik and Morrison (2022). The textiles were ‘naturally’ contaminated over time by indoor air with known PCB contamination, so the PCBs were not incorporated into the textile fibres. Washing removed up to 84% of sorbed PCBs, with a median of 67%. A similar decrease in concentrations was observed in some samples in our study for CPs.

The stronger changes in some samples after washing could indicate that the CPs were not directly incorporated into all fibres, but were also used as additives in dyes or originated from external contamination (adsorbed on the surface of the tested material or as part of dust particles). The second possibility is also supported by the fact that some samples can become contaminated during the washing process. Two ways of cross-contamination are possible: contamination of the washing water with subsequent adsorption on the samples or the release of microfibres rubbed off textile samples with higher SCCP and MCCP levels. Also, contamination by components of the washing machine cannot be excluded without further investigations.

Looking at the CP profiles, washing led on average to slight changes in the CP profiles (Figure 2). The profiles of pure cotton samples, which were more susceptible to contamination (partly due to lower initial CP contents), shifted (for both SCCPs and MCCPs) towards congener groups with fewer C and Cl atoms. These congener groups are more likely to dissolve in water and therefore allow cross-contamination during the washing process. On the other hand, the profiles of samples with a high proportion of synthetic fibres (which on average emitted the CPs) shifted to congener groups with more C and Cl atoms, supporting these conclusions.

5. Conclusions:
Not surprisingly, a strong influence of the material on the CP contents was found – with higher CP contents in samples made from synthetic fibres than in samples made exclusively from cotton. In addition, the effect of washing on the tested clothing was investigated. When all samples were considered, the washing process resulted in significant changes. When looking at individual samples, some samples were found to emit excessive CPs, others were contaminated by the washing process and still others were able to maintain their CP contents. The behaviour was somehow unpredictable in this first pilot test. Samples with values close to the LOQs were able to release CPs, while the sample with the highest SCCPs concentration was able to maintain its value. The changes in concentrations were accompanied by changes in the profiles of CPs – sometimes in association between groups. The shift in profiles from pure cotton samples towards congener groups with fewer C and Cl atoms after washing was compensated.
by an opposite trend in samples with a significant proportion of synthetic fibres – indicating the possibility of cross-contamination. The SCCP and MCCP contents in clothing, although significantly lower than those reported in the literature for polymeric products, could still have some negative consequences. Clothing is in direct contact with human skin for long periods during the day and can be a source (through abrasion) of (micro)fibres that become part of house dust – an important route for human exposure to various pollutants. Last but not least, the possibility of CPs being released during the washing process could be an important emission pathway into the environment. In the future, dermal exposure through clothing and the effects of washing should be further investigated.

6. Acknowledgments:
This work was financially supported from the grants of Specific university research – grant No. A2_FPBT_2020_041 and A1_FPBT_2023_002. The support from the Czech Science Foundation (21-19437S) is also gratefully acknowledged.

7. References:
14. UNEP (2022). Risk profile for chlorinated paraffins with carbon chain lengths in the range C14–17 and chlorination levels at or exceeding 45 per cent chlorine by weight.
Introduction: Chlorinated paraffins (CPs) are widely used in metalworking, flame retardants, and plasticizers, and have become a major environmental concern due to their high production volume, persistence, and bioaccumulation[1]. Long-term exposure to short, medium, and long-chain CPs has been associated with adverse health effects in humans. Personal exposure to CPs occurs via different pathways, with dermal contact and dust ingestion being important routes. Settled dust and hand-wipes are used to assess exposure, but they cannot effectively reflect the whole personal exposure scenario. Silicone wristbands (SWBs) are considered an emerging sampler for personal external exposure assessment due to their non-invasive nature, high diffusivity, strong hydrophobicity, low reactivity, high flexibility in shape, and affordable price[2]. This pilot study aims to evaluate the potential use of SWBs as a personal sampling tool to monitor human exposure to CPs in the indoor environment. The study objectives include measuring the concentrations of SCCPs, MCCPs, and LCCPs (C18-20) in SWBs, exploring the CP homolog patterns in the samplers, and estimating personal exposure via dermal contact based on the theoretical bio-accessibility fraction of each CP class.

Materials and Methods: The SWBs were purchased online, cleaned, and then deployed on 12 volunteers during July and August 2022, who wore the SWBs continuously for a week. Three SWBs were also used as stationary field samplers (FSs) to monitor the environment for CP contamination. The SWBs were then extracted and analyzed for CPs using an Agilent 1290 Infinity II – 6560 Q-TOF MS. The sample preparation was based on methods developed and in-house validated for CP analysis in dust samples[3], with internal and external standards used during the extraction process.

Results: The results of the study show that the quantifiable CP classes, including SCCPs, MCCPs, and LCCPs (C18-20), were detected in all worn SWBs. The median concentrations of ∑SCCPs, ∑MCCPs, and ∑LCCPs (C18-20) in worn SWBs were 19 ng/g wb, 110 ng/g wb, and 13 ng/g wb, respectively. Median concentrations of ∑SCCPs, ∑MCCPs, and ∑LCCPs (C18-20) in the three FSs were 1.2 ng/g wb, 2.8 ng/g wb, and <LOQ, respectively. Higher lipid contents were found in worn SWBs than in the FSs. The concentrations of CPs were significantly different between the worn SWBs and FSs in both mass and lipid-weigh basis, while for the chlorine content, the difference was not significant. The ∑FSs concentrations were found to have significantly lower ∑MCCPs, but not of ∑SCCPs compared to the worn SWBs, indicating that the exposure assessment using field samplers could underestimate the MCCP exposure from the environment to the participants by one to two orders of magnitude. Among the worn SWBs and FSs, the median ∑SCCP/∑MCCP (S/M) were 0.26 and 0.61, respectively.

Discussion and Conclusion: Lipophilic chemicals in human biomonitoring surveys are usually reported in lipid-adjusted concentrations to allow a more accurate comparison between the same/different matrices with different lipid ratios. The median SCCP/MCCP ratio was higher in FSs than SWBs, indicating that the exposure assessment using field samplers could underestimate the MCCP exposure from the environment to the participants. The exposure to CPs was found to be related to the micro-environment contamination while outliers showed that other sources of exposure are likely to exist. CP exposure poses a nonnegligible risk to humans via dermal contact based on the estimation of exposure in the worst-case scenarios. Results presented in this pilot study provide a proof of concept of using SWBs as a cheap and non-invasive personal sampler in future studies focusing on CPs.

Acknowledgments: FWO junior post-doc fellowships for S. Yin and T. J. McGrath (1270521N/12Z9320N), National Natural Science Foundation of China (22276166), and the Exposome Centre of Excellence of the University of Antwerp (41222).

References:
Fate, Detection and Analysis of Chlorinated Paraffins

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1. Introduction:
Chemical pollution, particularly from Persistent, Bioaccumulative, and Toxic (PBT) chemicals like polychlorinated biphenyls (PCBs), poses extensive and enduring health risks for humans and ecosystems worldwide (Dewailly et al., 1993). These impacts persist even decades after their near-global ban (Desforges et al., 2018). The Stockholm Convention mitigated the effects of PCBs and similar Persistent Organic Pollutants (POPs). However, the substitution of PCBs with related chemicals, specifically chlorinated paraffins (CPs, chemical formula: CnH2n+2-mClm), has presented new concerns for human and environmental health (Wang et al., 2022).

Recent studies have found CPs globally, including in human mothers' milk, indicating pervasive exposure (Krätschmer et al., 2021). Adverse effects include developmental toxicity and endocrine disruption (Sprengel et al., 2021). With short-chain CPs (SCCPs) now under global restriction, there is an increasing focus on medium- (MCCPs) and long-chain CPs (LCCPs) (POPRC, 2021). The current study hypothesizes that the various exposure pathways of CPs contribute significantly to the body burdens of these chemicals in humans. To test this, the research aims to comprehensively quantify these exposure pathways and assess their relative contributions using a one-compartment pharmacokinetic model and a forensic fingerprinting approach. The findings will have substantial implications for developing effective management strategies to protect human and ecosystem health.

2. Materials and Methods:
This study involved the collection of samples from dust, air, diet, and hand wipes to examine the exposure levels of SCCPs, MCCPs, and LCCPs among 61 adult participants (45 women and 16 men with ages ranging from 20 to 66) from Oslo, Norway between 2013 and 2014. Samples were collected using hand wipes, high-volume air samplers, dust sampling kits, and duplicate diet collections (Papadopoulou et al., 2016). The Regional Committees for Medical and Health Research Ethics in Norway granted approval for this study (Case number 2013/1269), with all participants providing written informed consent before participation. The chemical analyses conducted in Sweden also received the necessary approval from the Regional Ethics Committee in Stockholm (Case number 2014/624-31/1).

For analysis, an Ultra-High-Performance Liquid Chromatograph (UPLC) combined with a Quadrupole-Orbitrap ultra-high resolution mass spectrometer (Orbitrap-MS) was employed, with atmospheric-pressure chemical ionization (APCI) for the ion source (Yuan et al., 2021). Hand wipe samples were analyzed using a direct-injection Orbitrap-MS method (Yuan et al., 2020). Quantification was executed via a CnH2n+2-mClm-profile deconvolution method with 21 reference mixtures (Du et al., 2020). Exposure was calculated based on dietary intake, dermal exposure, inhalation, and dust ingestion, and plasma concentrations were estimated using a one-compartment, first-order pharmacokinetic model (Tay et al., 2019). The model uses a compound-specific dissipation rate and considers each individual's body lipid mass. Statistical analysis was applied to compare CP amounts in samples and assess correlations with variables in the indoor environment.

A forensic fingerprinting method assessed contamination sources through a CnH2n+2-mClm-profile (Yuan et al., 2017). When multiple contamination sources were present, deconvolution allowed for the assessment of each source's relative contribution. The procedure included considerations of bioaccessibility for CP homologues and aimed to achieve a high goodness-of-fit between the plasma profile and the superimposed profile.

3. Results and Discussion:
The cohort participants were found to be exposed to an intricate mixture of CPs that exhibited a wide spectrum of carbon chain lengths, extending from C6 up to C48. The majority of these CPs contained a moderate number of chlorine substitutes, primarily within the Cl5 to Cl8 range. However, a dust sample showed the presence of highly chlorinated homologues of CPs, specifically those with chlorine content nearing saturation levels, from CnCln-2 to CnCln+2.

CPs emerged as the predominant flame retardants within the Norwegian cohort, according to our study. Various organic chemicals were examined across the same cohort sample sets in previous studies (Tay et al., 2019; Xu et al., 2019). These sample sets revealed a substantial presence of CPs, suggesting significant human exposure to this commonly used chemical. Conversely, organophosphate esters (OPEs) were more abundant in stationary air samples, possibly due to their differing volatilities.
In the cohort’s diet, dust, and hand wipe samples, MCCPs were the most common CPs, making up 64%, 56%, and 58% of total CP concentrations, respectively. SCCPs, the legacy CPs, were predominant in diet samples and air, and LCCPs were also significant in dust and hand wipes. Estimated daily exposure to CPs were shown in Table 1. The median exposure to SCCPs, MCCPs, and LCCPs was 61, 69, and 2.8 ng/kg bw/d, respectively, and the 95th percentiles of exposure were 190, 190, and 16 ng/kg bw/d. These levels are considerably below the oral reference doses of 2300, 6000 (EFSA, 2019), and 71000 ng/kg bw/d for SCCPs, MCCPs, and LCCPs, respectively. Dietary exposure accounted for 60-88% of total CP intake. Inhalation accounted for 7% of SCCP intake, while dermal exposure was the second most significant exposure pathway for MCCPs (14%) and LCCPs (29%). Dust ingestion’s contribution to total human exposure increased with CPs of varying chain lengths. It accounted for 2.2% of the total exposure for SCCPs, 3.3% for MCCPs, and increased to 10% for LCCPs.

In the plasma samples of the participants, CPs were detected in concentrations ranging from below the limits of detection up to 91 ng/g wet weight, with a median concentration observed at 24 ng/g wet weight. When evaluated on the basis of fat weight, the median and maximum concentrations of CPs were 4200 ng/g lipid and 21,000 ng/g lipid, respectively. SCCPs were found to be the most prevalent, constituting a median of 66% of the total CPs. MCCPs and LCCPs accounted for median contributions of 29% and 3.2%, respectively. Although the median plasma levels of legacy SCCPs were lower than those reported in China, the median levels of current-use MCCPs and LCCPs were comparable (Yuan et al., 2022). Significant correlations were found between concentrations of SCCPs, MCCPs, and LCCPs in plasma and dietary intake, but not with other exposure media. Personal air samples showed a higher correlation with plasma concentrations than stationary air samples. Using the PK model, predicted plasma concentrations were compared with measured concentrations; the model predicted SCCPs accurately, while underestimating MCCPs and LCCPs. The underestimation could be due to not all exposure pathways being identified and included in the model, as well as potential inaccuracies in the absorption fraction of LCCPs and the assumed steady-state condition.

Table 1: Estimated Daily Exposure to CPs for Adults (ng/kg bw/d)

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Percentile</th>
<th>SCCPs</th>
<th>MCCPs</th>
<th>LCCPs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary</td>
<td>50th</td>
<td>42</td>
<td>96</td>
<td>8.5</td>
</tr>
<tr>
<td></td>
<td>95th</td>
<td>120</td>
<td>250</td>
<td>38</td>
</tr>
<tr>
<td>Dermal</td>
<td>50th</td>
<td>0.62</td>
<td>2.4</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>95th</td>
<td>3.6</td>
<td>11</td>
<td>5.5</td>
</tr>
<tr>
<td>Inhalationa</td>
<td>50th</td>
<td>8.8</td>
<td>1.1</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>95th</td>
<td>21</td>
<td>7.2</td>
<td>0.65</td>
</tr>
<tr>
<td>Dust ingestionb</td>
<td>50th</td>
<td>1.1</td>
<td>0.70</td>
<td>0.090</td>
</tr>
<tr>
<td></td>
<td>95th</td>
<td>7.9</td>
<td>4.1</td>
<td>0.60</td>
</tr>
</tbody>
</table>

| a) Based on stationary air results. |
| b) Based on settled dust results. |

Dietary intake was the primary exposure pathway to SCCPs, MCCPs, and LCCPs in the study, with inhalation, dust ingestion, and dermal exposure also contributing, though to a lesser extent. The individual contribution of each pathway varied among participants. A forensic fingerprinting approach was employed (Figure 1), which entailed reconstructing the chemical fingerprint of each participant’s plasma sample using samples from various exposure pathways. This confirmed that diet contributed most to exposure, with inhalation exposure decreasing from SCCPs to LCCPs, while dust ingestion and dermal exposure played a more significant role for LCCPs. Notably, the fingerprinting approach highlighted potential over- or underestimation of intake contributions, necessitating further exploration of personalized sampling strategies. Personal air samples showed a higher correlation with plasma samples than stationary air samples, suggesting their potential utility in refining exposure estimates.
4. Conclusions:
This study found diet as the primary exposure pathway for CPs in the Norwegian cohort, with inhalation, dust ingestion, and dermal exposure contributing to a lesser extent. Predicted plasma concentrations of CPs were generally accurate for SCCPs, but underestimated for MCPPs and LCCPs, suggesting unidentified exposure pathways or model inaccuracies. The study also showcases the potential of a forensic fingerprinting approach in the examination of complex mixtures like CPs. It provides a more nuanced understanding of individual exposure and contribution of each pathway and underscores the necessity for more individualized sampling methodologies and enhanced models for exposure pathways, which are crucial for precise risk assessments. There is an urgent need for the development of efficient management strategies specifically tailored towards the currently used MCPPs and LCCPs, to ensure the health and safety of individuals and ecosystems alike.

5. Acknowledgments:
Wanjiao Kong (SU) is acknowledged for sample preparation and data processing of diet and plasma samples. All participants are acknowledged for their contribution. We would also like to acknowledge A-TEAM’s PhD fellows for their contribution during the A-TEAM sampling campaign. The research leading to these results has received funding from the European Union Seventh Framework Programme FP7/2007–2013 for research, technological development, and demonstration under grant agreement number 316665 (A-TEAM project). The CP analyses were funded by the Nordic Council of Ministers (project number 2019-008). For detailed methods and discussion see Yuan et al. (2023).

6. References:

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**Figure 1:** Forensic fingerprinting CP homologues in a plasma sample. The vertical axis is the relative abundance of CnClm. The horizontal axis lists the CnClm from C10 as the low detection frequencies of vSCCPs in multiple matrices. Modified from Yuan et al. (2022).
Fate, Detection and Analysis of Chlorinated Paraffins
M.Ricci & A.Fernandes

TUE-AM-D5  Human Exposure to Chlorinated Paraffins: A Norwegian Cohort Study

**Introduction:** The characterization of polychlorinated alkanes (PCAs) is hampered by major analytical challenges that remain unresolved to this day. Although the chemical formula remains relatively simple ($C_nH_{2n+2-x}Cl_x$), tens/hundreds of thousands isomers may arise from the industrially process based on UV-initiated radical chlorination from nalkane mixtures. The most advanced analytical strategies are based on the combination of chromatography with high-resolution mass spectrometry after soft ionization of the analytes, i.e. not generating excessive mass fragmentation. The approach allows to deal with significant mass interferences within the chemical group[1]. Thus, at best, full-scan HRMS datasets allow the study of homologue pattern, while chromatographic traces remain as unresolved isotopomer humps. So far, each research group has its own laborious workflow based for integrating hundred/thousands of signals[2,3]. Here we present CP-Seeker, a portable and user-friendly application aiming at processing complex HRMS datasets to integrate and visualize signals arising not only from PCAs but also from related alkenes, putative metabolites or bromo-chloro analogs.

**Materials and Methods:** CP-Seeker works without the need for computer skills or administrative rights. An embedded web-browser interface (*Shiny* framework) commands the *R* functions. After importing the data files (Orbitrap or ToF), the proprietary raw data are converted into the open format mzXML using MSConvert software. For the deconvolution, the user can then select among several chemical families (PCAs, brominated analogs – PBAs, mono- to triene analogs – PCOs, phase I and phase II putative metabolites, bromo-chloro analogs – PXAs) and several negative ion types ([M-H]-, [M+Cl]-, [M+C$_2$H$_3$O$_2$]-, [M+Br]- for ESI/APCI-based techniques; [M-Cl]-, [M-HCl]-, [M-Br]-, [M-HBr]- for ECNI-based techniques). A range of internal standards is also available. The user also selects the mass tolerance, the mass analyzer nature and nominal resolution, as well as a few other parameters. Once launched, the script automatically reconstitutes the isotopic pattern of each homologue group ranging from C$_6$ to C$_{36}$ and from X$_3$ to X$_{30}$ ($X=Cl$ and/or Br) observed within the acquired $m/z$ range, according to the theoretical pattern to be observed with the mass analyzer (isotopomer groups above 1% of the base peak, enviPat package[4]). Homologue groups with $n Cl > n C + 3$ are excluded. The script is designed to efficiently integrate non-Gaussian chromatographic humps. Areas are normalized to the theoretical sum of peaks. Once achieved, the user can review matrix tables providing the intensity, the pattern score and the mass deviation for each homologue group in each sample and for each selected adduct. A click on a cell displays corresponding EICs and the head-to-tail comparison of reconstituted and theoretical mass spectra. Data can be exported as csv files and formatted Excel spreadsheets.

**Results:** Figure 1 illustrates some tabs of the CP-Seeker software.

![Figure 1: Screenshots of the CP-Seeker interface.](image)

**Discussion and Conclusion:** CP-Seeker is meant to be freely available, under the CC-BY 4.0 license (contact.cpseeker@oniris-nantes.fr).

**References:**
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Introduction: The prime threats perceived to contribute to the global decline of shorebirds include climate change and habitat loss, while the role of pollution in these declines has been less investigated. Whereas the rate of destruction of natural wetland habitats has been particularly rapid over the past decades, artificial wetlands, including wastewater treatment plants, have seen an increase over this time. These new habitats, in turn, pose a pollution risk to wildlife (Arvaniti & Stasinakis, 2015). The extent of this threat to shorebirds has not yet been studied, and birds inhabiting artificial wetlands were expected to have higher pollution burden and poorer health and survival than those residing in more ‘natural’ wetlands.

Materials and Methods: We compared exposure to pollution by 15 per/polyfluoroalkyl substances (PFASs) and 15 elements along with prevalence of avian influenza, oxidative stress and local survival in two long-distance migratory shorebird species, curlew sandpipers (Calidris ferruginea) and red-necked stints (Calidris ruficollis). Birds were sampled for blood from 2011-2020, at two contrasting habitats on their Victoria, Australia non-breeding grounds: a natural wetland on Western Port Bay (WPB) and a putatively more polluted artificial wetland at Melbourne’s Western Treatment Plant (WTP), the latter of which processes the waste of approximately 5 million people.

Results: Blood pellet concentrations of both carboxylate PFASs and sulfonate PFASs were found to be significantly higher at the Western Treatment Plant (carboxylate PFASs WTP median: 15.7 ng/g, range: <0.01-107 ng/g; WPB: 2.05 ng/g, <0.01-62.3 ng/g - sulfonate PFASs WTP median: 65.3 ng/g, range: <0.01-804 ng/g, WPB: 4.86 ng/g, <0.01-26.9 ng/g). Our results indicated limited significant site differences in blood pellet concentrations of elements except for mercury and selenium. However, Avian influenza prevalence was higher at the natural wetland, while seropositivity (representative of prior infections) was higher at the WTP. We also measured higher blood o,o'-dityrosine (an indicator of protein damage) at the WTP. No significant differences were found for adult survival, but survival of immature birds at the WTP appeared to be lower than those at the natural wetland (WPB).

Discussion and Conclusion: Our findings showed that PFASs from wastewater treatment plants indeed contaminate the birds inhabiting the region. However, concentrations in most individuals were lower compared to health risk thresholds (Dennis et al., 2011; Newsted, Jones, Coady, & Giesy, 2005). These relatively low concentrations, together with limited other significant differences in pollution and health indicators, may suggest that appropriately managed wastewater treatment wetlands may provide an alternative habitat to these migratory species. In the face of widespread habitat destruction, these artificial wetlands may prove critical in curbing the decline of shorebird populations.

References:
**Introduction:** Anthropogenically induced chemical pollution of the aquatic environment is ubiquitous. Even though the use and production of many pollutants is restricted in Europe, high concentrations remain present in the environment due to their persistence. Therefore, it is imperative to continue monitoring these compounds. Persistent organic pollutants (POPs) can reach high accumulated concentrations in biota due to bioaccumulation and magnification. Within the Water Framework Directive, Environmental Quality Standards for biota (EQSbiota) were derived for strongly hydrophobic or lipophilic compounds (including PFOS, PBDEs, HBCD, mercury, dioxins) (European Commission, 2013).

**Material and methods:** An extensive ongoing monitoring campaign sampling 45 locations covering the main water bodies in the northern part of Belgium (Flanders) allowed for the monitoring of these compounds between 2015 and 2023. Concentrations were analysed in the muscle tissue of European perch (*Perca fluviatilis*) and European eel (*Anguilla anguilla*). Additionally, PCBs and CPs were analysed as well. Each sampling location was sampled every 3 to 4 years.

**Results:** The existing network provides an elaborate dataset that allows for temporal analysis of pollutant concentrations in predatory fish species, reflecting the local pollution load.

**Discussion: and Conclusion:** The results of the present study will create a better insight in the environmental presence and degradation of environmentally persistent pollution even after action (i.e. reduction or restriction of the use and production) has been taken. This might give an indication if additional action is needed.

**Acknowledgements:**
This study was partially funded by the Flanders Environment Agency. The technical crew of INBO Linkebeek is acknowledged for their help in fish sampling.

**References:**
Introduction: European eel (Anguilla anguilla) is professionally fished and marketed in the Netherlands. Persistent organic pollutants (POPs) such as polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs), polychlorinated biphenyls (PCBs) and perfluoralkyl substances (PFASs) accumulate in eel. In the Netherlands, a monitoring program was installed since late 1970’s on the monitoring of POPs in inland waterways. Eel was chosen as the target species as it is widely distributed in these waterways, and the high lipid content in eel and high POP levels facilitated the detection of POPs. Previous research showed that eel from various locations does not comply with the EU maximum levels (MLs) for PCDD/Fs and PCBs (EU Regulation 1881/2006) [1] and presented a risk for the consumer. Therefore, since 2011, major rivers in the Netherlands are closed for commercial fishing [2]. In other waters such as lake IJssel eel fishing is still allowed, as levels are substantially lower. It is important to investigate time trends in eel from these areas to determine whether the PCDD/F and PCB levels in eel have decreased. Here we present the temporal trends of PCDD/Fs and PCBs in eel from the Netherlands from 2012 to 2022. Next to this, we report on PFASs levels in eel from these locations, and on their trends from 2017 to 2022.

Materials and Methods: Eel samples were annually collected on 8 different trend locations of inland waterways and additional locations (varying every year) in the Netherlands. From every location, filets of ideally at least 15 individual fish were pooled resulting in 1 pooled sample. The sampled eel were in the size range of 53-76 cm (before 2016 >45 cm), according to the recommendations in Kotterman et al. [3]. PCDD/Fs and dl-PCBs, ndl-PCBs and PFASs were analyzed in these eel samples. Details on the chemical analysis can be found elsewhere [1]. The levels of PCDD/Fs and PCBs were evaluated against the MLs [1].

Results: Temporal trends could be established for PCDD/Fs and PCBs in eel on 8 trend locations from 2012 to 2022, and for PFASs from 2017 to 2022. In general, the highest levels of PCDD/Fs and PCBs (WHO2005-PCDD/F-PCB-TEQ of 10-26 pg/g ww) were measured in eel from closed fishing areas such as the major rivers (Meuse, Waal, Rhine and IJssel) and the lower river delta area (among others Hollands Diep and Volkerak). Lower levels of PCDD/Fs and PCBs (WHO2005-PCDD/F-PCB-TEQ of 3-5 pg/g ww) were found in lake IJssel, and other more remote areas (i.e. less affected by the pollution from major rivers). In terms of PFASs, mainly PFOS, PFNA, PFDA, PFUnDA and PFDoDA were detected, with PFOS dominating in all samples (2 – 30 µg/kg ww).

Discussion and Conclusion: For most locations, PCDD/F and PCB levels do not show a clear upward or downward trend over the period 2012-2022. On a fat weight basis, the PCDD/F and PCB levels are even more stable over the years. It is unlikely this will change in the near future when the contamination in river sediments will remain stable as well. PFAS levels showed a different distribution over the rivers, and levels observed in remote locations were in some cases similar to levels found in major rivers. Most likely this is because PFASs originate from different sources, and have different chemical properties (and thereby different distribution patterns) than PCDD/Fs and PCBs. In terms of PFAS temporal trends, some locations show a downward trend in PFAS levels over the period 2017-2022 which suggests changes in the environmental levels.

Acknowledgments: The Dutch Ministry of Agriculture, Nature and Food Quality is gratefully acknowledged for financial support of the study (WOT-02-001-014). The technicians taking care of sampling and chemical analysis, are gratefully acknowledged.

References:
1. Leenders et al. (2022). “Contaminanten in rode aal uit Nederlandse binnenwateren; Resultaten uit 2021” WFSR-rapport 2022.014. (in Dutch)
2. Implementation regulation regarding fishery by the Dutch government (https://wetten.overheid.nl/BWBR0024539) (in Dutch)
TUE-AM-E4 Distribution and Concentration Of PCDD/PCDF In Emerita Analoga Colected On The Central Coast Of Chile. South America

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1 Laboratorio de Oceanografía Química, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Concepción, Chile, Postal code 4070386, rodrigoloyola@udec.cl.

Polychlorinated dibenzo-p-dioxins (PCDD) and polychlorinated dibenzo-p-furans (PCDF) are widely distributed in the environment. The sources of these compounds may have different sources and once being released to the environment are finally bioconcentrated in organisms, specially those with high content of fat. Emerita analoga is a decapod crustacean that lives in the coast of the pacific Sea, that organism is widely distributed on the coast of Chile, specially in central Chile. In some areas of Chilean coast, emergita analoga is used as food. The concentrations of PCDD and PCDF were measured in a long-term monitoring program in emergita analoga collected on the coastal area of the region del Bio bio, Chile. Samples were carried to the laboratory then lyophilized and homogenized. The extraction and purification of the samples was performed using pressurized liquid extraction (PLE) and Power Prep system (FMS.Inc). The quantification of PCDD and PCDF were carried out by isotopic dilution using a HRGC-HRMS Thermo DFS. The results shows high concentration of PCDD/PCDF, specially the congeners OCDD, OCDF, HpCDD and HpCDF. Additionally the concentration show a seasonal pattern that may be related with the reproductive cycle of the organism and the variation in the content of fat.

Acknowledgements:
Authors acknowledge to the administration of the "Laboratorio de Oceanografía Química" of Universidad de Concepcion, for their financial support to carry out this research. The staffs of the laboratory are thanked to their contribution in the acquisition of chemicals supplies that allowed extraction, analysis and sampling.
1. Introduction:

Human activities, pollution and climate change are causing significant alterations of marine ecosystems although sea is increasingly attracting interest as sustainable supply of natural resources. Monitoring the "state of health" of coastal waters ecosystems is of primary interest. Mussels, as a result of their ability to filter large volumes of water and concentrate contaminants, have been used as "pollutants sentinels" since long time and all over the world, besides being a relevant food resource. Chemicals widely produced because of their technological properties, often turned out to be threatening substances, as a results of their persistence and toxicological properties [1]. Some classes, like dioxins and polycyclic biphenyls, have already been deeply investigated, while others still need scientists' efforts to elucidate toxicity mechanisms, accumulation and biotransformation abilities, and levels in food and environment. Brominated flame retardants (BFRs) and perfluoroalkyl substances (PFASs) are considered emerging persistent organic contaminants (POPs). BFRs have been used as additives in a wide variety of commercial and industrial products to inhibit or delay the spread of fire. The oldest and most widely used were polybromodiphenyl ethers (PBDEs) and hexabromocyclododecanes (HBCDs) [2], which were banned by the Stockholm Convention [3-6]. Concerns have been raised regarding their potential dioxin-like toxicological properties and their endocrine-disrupting effects [7-9]. As a result of these bans, the use of newly synthetized flame retardants, classified as "emerging" (eBFRs), is increasing. Members of this group are Bis (2,4,6-tribromophenoxy)ethane (BTBPE), decabromodiphenylethane (DBDPE), Hexabromobenzene (HBBz), pentabromobenzene (PBBz), pentabromomethylbenzene (PBE), pentabromotoluene (PBT), hexabromocyclopentenyldibromocyclooctane (HCBDCO), 2-ethylhexyl 2,3,4,5-tetrabromobenzoate (EHTBB) and 2,3,5,6-tetrabromo-pxylene (pTBX). Following their increasing use, these compounds have been detected in environmental and food matrices. It has been proven that many eBFRs have properties similar to POPs, rising human health concerns [10].

PFASs, highly fluorinated aliphatic compounds, have been largely synthesized and used in many industrial applications [11]: as a result of their resistance to degradation, they are widely detected in the environment. They turned out to be toxic, persistent, and bio-accumulative in biota and humans [12]. PFOS (perfluorooctanesulfonic acid), PFOA (perfluorooctanoic acid) and PFHxS (perfluorohexane sulfonic acid), were listed or recommended for listing in the Stockholm Convention [13-14]. In January 2023 maximum levels for PFASs (PFOS, PFOA, PFNA, PFHxS and their sum) in food (including mussels) were set [15].

The present paper reports the preliminary results of an extensive monitoring of the above listed emerging POPs in mussels (Mytilus galloprovincialis) collected along the coast, all over Italy, from Venice to Sicily and from Sicily to Genoa, trying to highlight differences and critical hotspots.

2. Materials and Methods:

2.1 Sampling

The sampling campaign was conducted by the network of the Italian Istituti Zootecnici Sperimentali (IZSs) in one year time frame (February 2022 to January 2023). A single sampling point was selected in each region (totally 10 along the Italian coasts) and about one sample of mussels (Mytilus galloprovincialis) per month was collected from each point. To date 84 samples were analyzed (Fig.1). Each sample was a pool of commercial size specimens to reach roughly 200-500 g of mussel pulp sent to the IZS dell’Umbria e delle Marche for POPs analysis.

2.2 Brominated flame retardants analytical method

Nine BDE congeners (28, 47, 49, 99, 100, 153, 154, 183, 209), 3 HBCD isomers (a, b and g) and 9 eBFRs (pTBX, PBBz, PBT, PBE, HBBz, EHTBB, HCBDCO, BTBPE and DBDPE) were analyzed in isotopic dilution, using a single sample preparation followed by a dual detection in GC– (PBDEs and eBFRs) and LC-MS/MS (HBCDs).

The analytical method for PBDEs and HBCDs analysis was already thoroughly described [16]. The same conditions were applied with good results to the analysis of the eBFRs. Mussel samples (20 g) were subjected to QuEChERS-like extraction and to a double clean-up on acidified Extrelut NT-3/ SPE Si 1g/6mL tandem columns assembly followed by gel permeation chromatography. Each purified extract was divided into two fractions and reduced to dryness. PBDEs/eBFRs fraction was analyzed in GC-MS/MS on a 7890A GC – 7000B triple-quadrupole mass analyser (Agilent Technologies, Palo Alto, California, U.S.).
The HBCDs fraction was injected in a LC-MS/MS ACQUITY I-Class Ultra Performance Liquid Chromatography/ Xevo TQ-S micro IVD system (Waters, Milford, Massachusetts, U.S.) [16].

2.3 Perfluoroalkyl substances analytical method
11 perfluoroalkyl carboxylic acids (PFCAs: PFBA, PFPeA, PFHxA, PFPeA, PFOA, PFNA, PFDA, PFUdA, PFDoA, PFTrDA, PFTeDA) and 8 perfluoroalkane sulfonic acids (PFSAs: L-PFBS, L-PFPeS, L-PFHxA, L-PFHpS, L-PFOS, L-PFNS, L-PFDS and L-PFDoS) were analysed in isotopic dilution as previously described [17]. Mussel samples (2 g) were extracted twice in ultrasound bath with acetonitrile and purified on a Strata X-AW SPE cartridge (200 mg/6 mL), eluting with ammonium hydroxide 2% in methanol and subjected to dispersive SPE with Envicarb® and acetic acid. The instrumental analysis was performed on an ACQUITY I-Class-LC/Xevo TQ-S micro IVD system in ESI negative mode [17].

2.4 Quality Assurance/Quality Control
Background contamination was carefully monitored at any stage of the analytical process as previously described, and a strict quality control was implemented in each batch for all the contaminants classes [16-17]. Limits of quantification (LOQs) were estimated on fortified samples: 0.005 mg/kg for PBDEs (209: 0.10 mg/kg), 0.010 mg/kg for HBCDs, eBFRs (BTBPE: 0.020 mg/kg; DBDPE: 0.10 mg/kg) and PFASs (PFBA: 0.20 mg/kg; 0.010 mg/kg for remaining 18 analytes). Regular participation in inter-calibration exercises organized by the EURL ensured external quality assurance.

3. Results:

eBFRs were not quantified in all the sample analyzed. The mean value for S9PBDEs lower bound (l.b.) in all the 84 samples analyzed was 0.062±0.071 mg/kg, with a maximum value of 0.51 mg/kg measured in only one sample collected on the Sardinia Coast (IZS-Sa). In the different sampling sites, S9PBDEs ranged between a mean of 0.027±0.019 mg/kg (IZS-AM, the lowest) and 0.16±0.02 mg/kg (IZS-LER, the highest) (Table 1). BDE-47, -99, -49 and -100 were the congeners most frequently quantified, accounting on average for 47%, 19% 14% and 12% of the total PBDEs respectively (Figure 2). BDE-209 was quantified in only one sample from IZS-Sa at 0.51 mg/kg.

S9HBCDs l.b. ranged between 0.014 (IZS-M, the lowest) and 0.42 mg/kg (IZS-PLV, the highest) with a mean value of 0.13±0.09 mg/kg (Table 1). a-HBCD was measured in all the samples and represents on average 96% of the total HBCDs, while g- and b- isomers were respectively quantified only in 17% and 7% of the samples (Figure 2). S19PFASs mean value was 0.16±0.14 mg/kg considering all the samples analyzed. The higher concentration was measured in one of the six samples collected from IZS-LER (0.64 mg/kg) (Table 1). The long-chain carboxylic acids (C12-C14) characterized the pattern of all the samples analyzed. PFTrDA was the prevalent compound, accounting for 40% of the total PFAS contamination, followed by PFTeDA (19%), PFDoA (15%) and PFOS (11%). PFOA was below LOQ in almost all the samples (Figure 2).
TUE-AM-E5  Brominated flame retardants (PBDEs, HBCDs and eBFRs) and perfluoroalkyl substances (PFASs) in mussels collected along the Italian coast

Table 1: Descriptive statistics: median, 25th and 75th percentile, maximum, minimum, standard deviation

<table>
<thead>
<tr>
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<th>75th</th>
<th>max</th>
<th>min</th>
<th>mean</th>
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<th>75th</th>
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<th>min</th>
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<tbody>
<tr>
<td></td>
<td>S9PBDEs l.b. (mg/kg)</td>
<td></td>
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Figure 2: PBDEs, HBCDs and PFASs contamination patterns

4. Discussion:
The results were described using the box plots reported in Figure 3. S9PBDEs median value in most of the sampling sites is close to the median calculated for all the samples (total median value - 0.045 mg/kg). Much higher levels were measured in all the mussels collected by IZS-LER, in the sampling point located on the Emilian coast (Adriatic Sea). Significantly higher than the total median were also the samples from the Ligurian coast (IZS-PLV) and from Sicily (IZS-Si), as well as those collected from IZS-LT. Comparable were the results of S3HBCDs: samples from IZS-LER were the most contaminated, followed by IZS-Si, -PLV and -LT. The mussels collected in the Venetian lagoon (IZS-Ve) show lower HBCDs concentration.

Mussels from the Emilian coast are confirmed to be the most contaminate also for S19PFASs, followed by the Ligurian samples. Interestingly, all the samples from IZS-LT have much lower concentrations than the total median, as well as the mussels collected from the Sardinia coast (IZS-Sa). The highest contamination of the mussels from IZS-LER can be explained by the proximity of the sampling point to the Po River mouth, the largest and most important Italian river which flows through Piemonte, Lombardia and Emilia Romagna, the most industrialized Italian regions. The samples from IZS-Si, among those with the higher concentration of contaminants, were collected in a small salt pond on the coast and separated from the see by a thin strip of sand (Lago Torre Faro).

In case of PFASs, the only class of contaminant for which maximum limits in mussels are fixed [15], the l.b. sum of the four regulated analytes (S4PFAS: PFOA, PFNA, PFHxS, PFOS) was below the maximum limits (5.0 mg/kg for the S4PFASs; 3.0, 0.70, 1.0 and 1.5 mg/kg for PFOS, PFOA, PFNA, PFHxS respectively) for all the samples analyzed. However S4PFASs mean (0.022 mg/kg) accounts only for 14 % of the mean total concentration estimated as S19PFASs (0.16 mg/kg - Figure 4). These findings raise the question whether the four PFASs chosen are the best contamination indicators for all food matrices [18]. Perhaps, the inclusion of few other PFASs as indicators in the sum, may enable a more accurate estimation.

An extensive monitoring of PBDEs in mussels collected along the Central Adriatic Sea was already published [19]. In the best of our knowledge, few other similar surveys were published in Italy. Giandomenico et al. (2013) reported a study on PBDEs in M. galloprovincialis sampled all over the Apulian coast (Southern Italy). Maximum total PBDE levels was 3.89 mg/kg dry weight (about 0.78 mg/kg considering an 80% of water content) found in mussels from the Ionian Sea. These results are higher than those found in samples from IZS-PB [20] in the frame of the present study. Only Chiesa et al. (2018) investigated PFASs and PBDEs in mussels (n=50) collected at the Milan fish market. PBDEs were found only in four samples, with a maximum level of 0.5 mg/kg for the SPBDEs, while SPFASs concentration span was n.d. – 91.8 mg/kg [21].
**TUE-AM-E5**  Brominated flame retardants (PBDEs, HBCDs and eBFRs) and perfluoroalkyl substances (PFASs) in mussels collected along the Italian coast

**Figure 3:** Box plots reporting HBCDs, PBDEs and PFASs levels in mussel collected along the Italian coasts (dark line: total samples median value; red cross: outliers)

**Figure 4:** Comparison between different method for PFASs sum calculation: S4 vs S19

**5. Conclusions:**
Eighty-four mussel samples collected along the Italian coast were analyzed for PFASs, PBDEs, HBCDs and eBFRs, with the aim of assessing both environmental pollution and food safety. This is the first report in Italy which includes all these classes of POPs in a mussel monitoring. eBFR were below quantification limits in all the sample analyzed. These preliminary results shows that mussels collected at the Emilian coast sampling point (Adriatic Sea) seems to have the higher levels for all the contaminants analyzed. High levels were measured also in the samples from Ligurian and Sicilian coast. All the samples analyzed were below the maximum limits for the four regulated PFASs. Is worthy of note that this monitoring is still ongoing: other samples should be analyzed and further statistic evaluation will be needed for a proper assessment of the results.
6. Acknowledgments:
The authors gratefully acknowledge financial support from the Italian Health Ministry: IZS PLV 13/21 RCS - IIZSS: il mare in rete.

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Introduction: The pollution of Perfluorinated alkyl substances (PFAS) in aquatic environments is a worldwide concern of which the ecological impact is still not well understood [1]. This knowledge gap goes along with monitoring and regulatory challenges. In Europe, environmental quality standards have only been set for a limited amount of PFAS, while thousands of different compounds are produced. Moreover, the current Biota Quality Standard (BQS) for PFOS (9.1 ng/g ww) was derived for the protection of human health and top predators and does not consider the impact on lower trophic communities [2]. In addition, the monitoring of PFAS is still mainly based on the abiotic environment which does not consider differences in bioavailability. The community structure of macroinvertebrates can reflect the cumulative impact of pollution events because some taxa are more sensitive to pollution than others [3]. Therefore, the aim of this research is to study the relationship between accumulated PFAS concentrations and the macroinvertebrate community responses and if this approach is suitable to derive threshold body burdens that are protective of ecological damage.

Materials and Methods: Benthic macroinvertebrates were sampled at 35 sites across Flanders, Belgium. For PFAS analysis the three most commonly abundant invertebrate taxa were selected: Chironomus sp., Asellus sp. and Gammarus sp.. Besides resident taxa, caged zebra mussels (D. polymorpha) were also exposed at 25 sites. The invertebrate community responses were assessed by calculating the Multimetric Index of Flanders (MMIF) [3]. To study the relationship between the accumulated PFAS levels and the maximum ecological response, 90th quantile regression analyses were performed. Threshold body burdens were derived based on two methods, (I) the 90th quantile regression line and (II) the 95th percentile of PFAS concentrations measured at locations with a good ecological quality (MMIF ≥ 0.7).

Discussion and Conclusion: First, mainly long-chain PFCAs were detected in both resident taxa and Zebra mussels. Therefore, it is strongly advised to prioritize those compounds in future monitoring programs. Secondly, as increasing PFAS concentrations in Zebra mussels were related to a decreasing ecological water quality, it can be concluded that translocated zebra mussels are a suitable biomonitoring tool to (I) predict the bioavailability of PFAS in aquatic environments and (II) to predict the ecological responses of macroinvertebrate communities. Threshold body burdens for mussels could be derived based on both methods and are comparable. Resident taxa on the other hand are found to be less appropriate for the prediction of the ecological effects of PFAS on aquatic ecosystems as no significant relationship was found. Despite this, threshold body burdens for resident taxa could still be derived for most compounds based on the 95th percentile method.

References:
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¹ MOE Key Laboratory of Pollution Processes and Environmental Criteria, College of Environmental Science and Engineering, Nankai University, Tianjin 300071, China

Introduction: Per- and polyfluoroalkyl substances (PFASs) have been used worldwide in multiple applications [1]. Crude oil production was acknowledged as a new source of PFAS to the environment [2], but the emission profiles from the downstream production activity remains unclear. This study investigated PFASs in the soil (n=45) from an oil refinery in Southwestern China. The contribution of unknown precursors was evaluated with total oxidizable precursor (TOP) [3]. Novel PFASs were identified by high-resolution mass spectrometry (HRMS) using an integrated nontarget approach of suspect and homologous screening based on fragment characteristics.

Materials and Methods: 1. Chemicals: 45 target standards 18 labeled internal standards of PFASs were used. 2. Sample preparation: 0.1% ammonia in methanol was used to extract soil samples. Samples were amended with potassium persulfate (K₂S₂O₈) NaOH for TOP assay. 3. Instrumental analysis: PFASs were performed with Agilent 1260 LC-6460 MS/MS (Agilent Technologies, USA) and Ultimate 3000 UPLC-Orbitrap-HRMS (Thermo Fisher Scientific, Germany) 4. PFAS screening: Compound Discoverer 3.3 were used for identification of features with typical fragments. Suspect and homologous screening were conducted accordingly.

Results: Thirty-five target PFASs were detected, The concentrations of ∑PFASs (C≥4) onsite (2.59-436 ng/g, median: 6.09 ng/g) were higher than those in background area (2.02-8.02 ng/L, median: 4.73 ng/g). With TOP assay, unknown precursors contributed 76-99 mol% to the profiles of PFASs in the soil of the refinery. In nontarget analysis, forty-eight PFASs homologues of eighteen classes were identified. H-substituted perfluoroalkyl carboxylic acids (HPFCAs, n=5-10) were identified as products of TOP assay. A possible short-chain component of aqueous film-forming foam, perfluoropropane sulfonamide was newly reported in refinery of China. Three alcohols were newly identified. Hexafluoro-2,2-propanediol, and C₆-cyclic-unsaturated perfluoroalkyl alcohol (C₆-CUPFA) produced after TOP assay were perfluorinated alcohols firstly reported in the environment. Hexafluorosopropanol was a polyfluorinated alcohol firstly identified as potential precursors for its absence after TOP assay. Bistriflimide (NTf₂) belonging to ionic liquids, was also first time reported in refinery-impacted environment.

Discussion and Conclusion: In TOP assay, the concentrations of 6:2 Cl-PFESA in soil samples were mostly reduced, although it was reported stable during TOP assay in Milli-Q water [4]. NTf₂ was used in solid-state supercapacitors and extraction agent as an end product, but only appeared in oxidized samples in this study, which needs verification with further study. Overall, the contamination profiles of PFASs in an oil refinery were clarified in this study, and new per/polyflurinated alcohols were identified and hexafluorosopropanol can be potential precursors for short-chain PFASs.

Acknowledgments:
This work was funded by the National Key Research and Development Program of China (2019YFC1804400), the National Natural Science Foundation of China (NSFC 22036004; 22006074), Tianjin Natural Science Foundation (19JCQNJC07400), and supported by Ministry of Education, China (T2017002).

References:
Introduction: Perfluoroalkyl substances (PFAS) are a group of commonly used compounds in industrial and consumer goods, known particularly for their hydrophobic, non-stick properties. Their unique chemistry led to their use in ski waxes. While regulations are changing for the manufacture of ski wax to exclude PFAS, the persistence of this class of compounds means they could still be detected for years. Given the hazards and global concern about PFAS contamination, we investigated if the contamination could be detected at a local ski area that supports a high-level race program. While previous studies have looked at targeted analysis for a set of known PFAS, this study used high-resolution mass spectrometry (HRMS) and ion mobility to look for new and unexpected PFAS.

Materials and Methods: Samples were collected from a variety of locations within a ski area in New Hampshire to investigate the trends of PFAS at this type of environment. The samples were acidified and extracted using weak anion exchange solid phase extraction cartridges to isolate and concentrate PFAS. A 22-minute LC gradient was employed on a 100 mm C18 analytical column using an LC that was modified to control for PFAS background contamination. Data collection was done with ion mobility enabled data-independent acquisition on a SELECT SERIES™ Cyclic™ IMS (Waters Corporation). Detected peaks were first compared to an internal HRMS PFAS library for identification. Unknown peaks were selected for further scrutiny based on their detected drift time in the ion mobility dimension.

Results: Data from each sample was acquired using ion mobility separation with full mass spectral acquisition under alternating high and low collision energy states. After acquisition, the data was componentized and processed against a PFAS library of ~100 compounds that included compound names, structures, retention time, and expected fragment ions. The added dimension of ion mobility can be used for spectral cleanup, which aids in the elucidation of unknown compounds as well as confirmation of known target compounds. In the extracts, several legacy PFAS including perfluoroalkyl sulfonates, perfluoroalkyl carboxylic acids, and fluorotelomer sulfonates were detected. A few emerging PFAS were also identified such as 11-chloroeicosafluoro-3-oxaundecane-1-sulfonic acid (11Cl-PF3OUdS). Identifications were based on accurate mass (<5ppm), identified fragments, and RT error. Observed collisional cross section (CCS) values were also compared to literature to confirm identifications.

Discussion and Conclusion: One challenge with non-targeted screening is being able to find unknown PFAS among thousands of detected features. Previous strategies for finding fluorinated compounds include Kendrick Mass Defect plots, common fragment searching, and mass defect filtering. For this study, we used ion mobility to select for potentially fluorinated or halogenated compounds. Previous studies have shown that the CCS values of per- and poly-fluorinated compounds are lower than compounds of similar m/z. An ion mobility filter was created based on this knowledge and applied to a list of detected peaks to select for possible PFAS compounds in a list of unknowns. Using this filter, a series (C9-C24) of polyfluorinated carboxylic acid compounds with one hydrogen substitution in the carbon chain were identified. The distribution for this series of compounds was not consistent, with longer chains found in samples from the base of the ski slopes and the shorter chains in the snowmaking retention pond. The observed CCS for this series was also compared with two different CCS prediction models.

Acknowledgments: Special thanks to Dartmouth College students: Isabelle Cheney, Vaishnavi Katragadda, Michael Chan, Daniel Chen, and Mateu Planelles for their assistance in sample collection and sample preparation.

References:
Per- and Polyfluorinated Substances (PFAS): Occurrence and Exposure
S.Voorspoels & D.Herzke

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Introduction: Per- and polyfluoroalkylated substances (PFAS) are a group of organohalogenated pollutants currently receiving much attention due to their ubiquity and proven negative effect on human health. In recent years, ski waxes have been identified as a potential source of PFAS in the environment. Commission Regulation 2020/784 came into force on July 4, 2020, which regulates perfluorooctanoic acid (PFOA) in selected products sold in the European Union, including ski waxes. The concentration of PFOA and their precursors should not exceed 25 ng/g of the product. Based on this regulation, the International Ski Federation banned the use of hydrofluorocarbon-based sliding waxes in international ski competitions starting winter season 2023-2024. However, recreational athletes use this type of wax without any restrictions. Although some ski wax manufacturers have stated that they have changed the chemical composition of their products, it was not confirmed by the chemical analysis.

Materials and Methods: 24 samples of cross-country skiing waxes were obtained from Czech biathlon clubs and the market network. 20 snow/water samples and 9 soil samples were collected in cross-country skiing areas during the Biathlon World Cup and regional biathlon competitions in the winter season of 2022/2023 in the Czech Republic. Isolation of PFAS from waxes was performed with ultrasonication using methanol. The snow processing procedure is based on the method for determining PFAS in water using SPE-Strata X-AW. Soil samples were extracted using acetonitrile solution followed by dispersive SPE purification. Target analytes included perfluoroalkyl carboxylic acids (PFCA, C4 to C18), perfluoroalkyl sulphonic acids (PFSA, C3 to C13) and fluorotelomer precursors. Analyses were done by ultra-performance liquid chromatography with tandem mass spectrometry (UHPLC-MS/MS) using electrospray ionization in negative mode.

Results: The concentrations of sum PFCAs (C4-C18) in cross-country skiing waxes ranged from 26 – 178 709 ng/g. PFOA concentrations in cross-country ski waxes ranged 9 – 40 625 ng/g. Individual wax samples therefore exceeded EU legislative regulation 2020/784. The snow samples mainly contained PFCA, the highest concentrations were measured during the Biathlon World Cup in Nove mesto na Morave. As part of this race, the total concentration of the amount of detected PFCAs (C4-C16) of 1113 ng/L was measured in the starting area. The PFCA concentration in the snow on the course of the race was in the range 54 – 633 ng/L. Analysis of water courses and soil in the vicinity of the cross-country skiing area showed the presence of PFAS. PFCA concentrations in watercourses during snowmelt ranged from 0.8 – 8.1 ng/L. Soil samples contained PFCA ranged from 1.5 - 130 ng/g dry matter.

Conclusion: The results of our study show a very high proportion of PFCAs in the snow and soil samples, with a profile corresponding to the findings in the wax samples. Which is an indicator that waxes with high PFAS content are still being used despite their restrictions. High PFCA concentrations in cross-country ski wax samples indicate minimal change in product composition.

Acknowledgments: This work was financially supported from a specific university research A2_FPBT_058 and A1_FPBT_2023_002.
Introduction:
Per- and polyfluoroalkyl substances (PFASs) are a group of synthetic chemicals that have been used in a variety of industries, including manufacturing, firefighting, and food packaging. These chemicals are persistent in the environment and can accumulate in the human body over time, which has led to concerns about their potential health effects. PFASs can enter the environment through industrial discharges, landfill leachate, and the use of firefighting foams. Once released, PFASs can travel long distances and persist in the environment for years. Thus, controlling PFAS emissions and lowering the risks to human health and the environment are both dependent on the presence of information about the contamination of waste-related samples. The likelihood of underestimating the total amount of PFASs discharged into the environment is high due to the limited knowledge regarding the presence of PFASs including perfluoroalkyl acids (PFAAs) precursors and their potential oxidative conversion products in various sources such as landfill leachate, firefighting foam, and materials coated with durable water repellent (DWR) products. Furthermore, these PFAAs precursors can break down into perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkane sulfonic acids (PFSAs) over time, and some of these fluorinated products may not be detected by conventional analytical methods. In addition, the lack of knowledge regarding PFASs including PFAAs precursors in waste-related samples has made the consideration of suitable PFASs emission control measures a major challenge.

DWR coatings based on side-chain fluorinated polymers (SFPs) are used to make water- or dirt-repellent clothing such as outdoor clothing, protective clothing for firefighters and medical services amongst other uses. DWRs can contain production-related known and unknown impurities which are PFAAs precursors. Consequently, several factors such as use, aging, washing and tumble drying of DWR coated clothing can result in PFASs including PFAAs precursors being emitted from the DWR to the environment. On the other hand, firefighting foams containing PFASs precursors are often discharged to the environment during response and/or trainings for response to emergencies. Fluorine-free firefighting foams (3F) have been developed for use as an alternative to firefighting foams containing legacy PFASs. However, these 3F foams contain short-chain C6 fluorochemicals manufactured using a telomer-based process and they have the potential to transform in the environment to shorter chain PFAAs which are also persistent, highly soluble, and highly mobile. Therefore, to determine the presence of PFASs including PFAA precursors and their oxidation products in firefighting foam, DWR, water- or dirt-repellent clothing and paper, we optimized the total oxidizable precursor (TOP) assay and the United States Environmental Protection Agency (USEPA) 3rd Draft Method 16333.

Materials and Methods:
Three firefighting foam samples (aqueous film forming foam (AFFF), protein foam (PF), and synthetic surfactant foam (SSF)) and two DWR samples (DWR-2011-2 and DWR-2011-5) were obtained for examining the use of TOP assay. All reagents used were of analytical grade except for water and methanol which were labelled “ultrapure water for PFOS and PFOA analysis” (GL Science, Japan) and “for water quality analysis” (Kanto Chemicals, Japan) respectively. Calibration standards were purchased from AccuStandard, USA and the Extractable Internal Standard (EIS) and Non-extractable Internal Standard (NIS) were purchased from Wellington Laboratories Inc., Canada.

Firefighting foam and DWR samples first underwent a rapid screening process, to ascertain sample volumes and dilution factors. For each sample, two aliquots were used. One aliquot followed the USEPA 3rd Draft Method 1633, however, slightly modified by concentrating the final extract to 1 mL (pre-TOP assay sample). Briefly, 20 µL of 50–1000 ng/mL EIS solution was added to a pre-determined sample volume (pH: 6.0–7.0) which was then loaded to a conditioned Strata PFAS (WAX/GCB), 200 mg/ 50mg/ 6 mL cartridges (Phenomenex, USA). This was eluted with 5 mL of 1.0% NH4OH in methanol, which also resulted in the carbon clean-up of the extracts as they passed through the SPE cartridges into a collection vessel. The extracts were concentrated to 1.0 mL using a TurboVap (Biotage, USA) (N2 (g) flow rate: 1.2 mL/min; water bath temperature: 55°C) prior to analysis on the Shimadzu LC-MS 8050 following the instrument settings in Table 1 and the ion, EIS and NIS conditions detailed in Table 7 of the USEPA 3rd Draft Method 16333. The presence of 40 PFASs was investigated in each sample.
The second aliquot (post-TOP assay sample) was subjected to our in-house TOP assay method which involved digesting 20 mL samples at 85°C for 12 h in 20 mL of 400 mM NaOH and 150 mM K2S2O8 mixed solution on a DigiPrep digestion block (SCP Science, Canada) (Figure 1). Our in-house TOP assay method is a modified version of the original Houtz and Sedlak, 2012\textsuperscript{2}) method which involved adding 60 mM K2S2O8 and 150 mM NaOH to 125 mL sample before digesting at 85°C for 6 h. Owing to the elevated pH (pH >12) and high temperature, reactions (1) and (2) (Figure 1) rapidly take place resulting in the formation of the hydroxyl radicals (∼OH); which oxidize PFAAs precursors to PFCAs. After digestion and adjusting the pH to pH: 6.0-7.0, the same SPE, concentration and analysis method as that of the pre-TOP assay samples was used. A comparison of pre- and post-TOP assay data was conducted to elucidate the presence of known and unknown PFASs including PFAA precursors in all samples investigated.

Figure 1: TOP assay process flowchart
Results:
Pre-TOP assay results for foam and DWR samples revealed that most PFCAs and PFSAs were below our laboratory’s lower detection limits for all analytes tested. For precursors, only 6:2 FTS was detected in the AFFF sample at nearly 470 mg/kg. Precursor concentrations in all other samples analyzed were below our laboratory’s lower detection limits. Post-TOP assay, C4–C10 PFCAs were the most dominant PFAAs in all samples except for PFDA which was detected in the DWR-2011-5 sample only, at a concentration of 80 mg/kg. 6:2 FTS concentration in the AFFF sample was below our laboratory’s lower detection limit, thus, it was assumed that 100% 6:2 FTS oxidation was achieved.

![Figure 2: Pre- and post-TOP assay results of PFASs in firefighting foam and DWR samples](image)

Furthermore, the EIS recoveries for all tested analytes in firefighting foam and DWR samples were within the Quality Control (QC) acceptance limits for EIS recoveries in wastewater samples stipulated in the USEPA 3rd Draft Method 16335 (Table 2).

**Table 2: Pre- and post-TOP assay EIS recoveries for firefighting foam and DWR samples**

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<tr>
<td>13C4-PFBA</td>
<td>101-111</td>
<td>15-78</td>
<td>10-130*</td>
</tr>
<tr>
<td>13C6-PFPeA</td>
<td>102-115</td>
<td>66-94</td>
<td>35-150</td>
</tr>
<tr>
<td>13C6-PFHxA</td>
<td>98-111</td>
<td>80-88</td>
<td>55-150</td>
</tr>
<tr>
<td>13C2-PFHxP</td>
<td>101-110</td>
<td>79-92</td>
<td>55-150</td>
</tr>
<tr>
<td>13C2-PFOA</td>
<td>96-121</td>
<td>85-98</td>
<td>60-140</td>
</tr>
<tr>
<td>13C2-PFNA</td>
<td>89-120</td>
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<td>87-111</td>
<td>78-95</td>
<td>50-140</td>
</tr>
<tr>
<td>13C2-PFUnA</td>
<td>88-108</td>
<td>76-84</td>
<td>30-140</td>
</tr>
<tr>
<td>13C2-PFDoA</td>
<td>77-111</td>
<td>72-98</td>
<td>10-150</td>
</tr>
<tr>
<td>13C2-PFTeDA</td>
<td>81-115</td>
<td>49-83</td>
<td>10-130*</td>
</tr>
<tr>
<td>13C2-PFBS</td>
<td>95-110</td>
<td>80-89</td>
<td>55-150</td>
</tr>
</tbody>
</table>

*In the multi-laboratory validation study data for wastewater matrices, some laboratories had difficulties achieving EIS recoveries in this range5*.
Per- and Polyfluorinated Substances (PFAS): Occurrence and Exposure
S. Voorspoels & D. Herzke

TUE-PM1-A4 Oxidizable conversion-based assessment of per- and polyfluoroalkyl substances (PFASs) in wastes and their related products

Discussion and Conclusion:
For firefighting foam and DWR samples, C4–C10 PFCAs were the most abundant compounds in the post-TOP assay results. The short chain PFCAs (C < 8) are highly mobile in the environment due to their increased water solubility and have the potential to accumulate in the edible parts of crops, thus, posing a great risk of PFAS exposure to humans and animals via diet. Since 6:2 FTS (whose post-oxidation products are C4–C7 PFCAs) was the only PFAA precursor detected pre-TOP assay in the AFFF sample, it is unquestionable that unidentified precursors in DWR, PF, SSF and AFFF samples may have contributed to the observed increase in the C4-C10 PFCAs concentrations post-TOP assay. These results concur with van der Veen et. al.’s findings that DWRs contain known and unknown impurities which are PFAAs precursors. Also, during the manufacturing process of AFFF and other firefighting foams, it is possible for PFASs including PFAAs precursors impurities to be introduced to the finished product through unreacted monomers, contaminants from raw materials and cross-contamination. Furthermore, some precursors and post-TOP assay transformation products may not have been identified due to the limited suite of analytes in the USEPA’s 3rd Draft Method 1633).

Results obtained in this study suggest that our in-house TOP assay method might be useful to elucidate the presence of PFAA precursors in the waste-related samples and to consider PFASs including PFAA precursors emissions comprehensively. Post-TOP assay oxidation products of each PFAA precursor and molar yields of PFCAs will be determined in future studies.

References:
Introduction:
Per- and polyfluoroalkyl substances (PFAS) are widely used in industrial and consumer products, such as water-repellent textiles and non-stick cookware. Unfortunately, some PFAS, e.g., perfluorooctanoic acid (PFOA), its salts and precursors have been regulated as persistent organic pollutants (POPs) under the Stockholm Convention. Nevertheless, there is a lack of research for understanding emissions of their PFAS to the environment, especially from waste treatment and recycling facilities. In our ongoing research, water repellents are considered to be one of the main emission sources from waste. In order to develop a screening method for estimating PFAS emission potential during two types of waste recycling processes; RPF (refuse derived paper and plastic densified fuel) manufacturing and thermochemical conversion from waste to syngas, we determined the amount of PFAS emitted during heating water repellents using a pyrolyzer gas chromatograph mass spectrometer (Pyro-GC/MS).

Materials and Methods:
The target PFAS were neutral 19 PFAS that included 13 POP-PFAS, such as 8:2 and 10:2 fluorotelomer alcohols (FTOHs), their acrylates (FTAC) and methacrylates (FTMAC), and so on. In this study, 10 commercial fluorotelomer-based water repellents (seven of the products were manufactured in 2011 and three in 2021) were identified and quantified by the following three analytical methods using a Pyro-GC/MS (pyrolyzer: PY-2020id [Frontier Lab] and GC/MS: QP2010 Plus [Shimadzu]): 1) Evolved Gas Analysis (EGA): Gas generated during heating a sample up to 600 ºC was directly injected into the mass spectrometer. This method can identify gaseous PFAS generated from a sample at elevated temperatures. 2) Desorption-Mode Analysis (DMA): PFAS vaporized while the pyrolyzer temperature was kept at a constant 250 ºC were quantified. 3) Pyrolysis-Mode Analysis (PMA): Gaseous PFAS discharged while pyrolyzing the sample at 600 ºC were determined. It was assumed that DMA and PMA would predict the potential emissions during RPF manufacture by heat-molding below 180 ºC and syngas production by pyrolysis over 600 ºC, respectively.

Results and Discussion:
In the EGA of water repellents, two fragments with [C3H3F2]+ and [C3H2F3]+ were observed over 300 ºC indicating fluorotelomer olefins (FTOs) and FTOHs were vaporized during heating the samples over 300 ºC. Therefore, a higher amount of such PFAS may have been released during pyrolyzing them.

In the DMA of water repellents manufactured in 2011, POP-PFAS, such as 8:2 and 10:2 FTOHs and their iodides (FTIs), FTACs and FTMarcs were detected. Their total emission potentials (mg) from 1 kg of a repellent ranged between 62 and 328 mg/kg (ppmw). It should be noted that waste recycling facilities for RPF production should pay attention for emissions of the detected POP-PFAS. Since the obtained emission potential is almost the same as the amount of impure PFAS found in the products in our previous work (Matsukami et al., 2022), the impurities may be responsible for the POP-PFAS emissions. On the other hand, for the two repellents manufactured in 2021, only the non-regulated 6:2 FTOH was determined; the emission potential from 56 to 180 mg/kg. This indicates that 6:2 FTOH is currently used as an alternative to POP-PFAS.

The PFAS emission potential determined by PMA was one or two orders of magnitude higher than by DMA. The significant release of FTOs and FTOHs is attributed to the thermal decomposition of ester bonds on the side-chains of fluorotelomer-based repellents. Therefore, pyrolyzing textiles and paper coated with PFAS-containing water-repellents to syngas should pay more attention on the large amount of POP-PFAS emissions. Finally, the reliability of DMA and PMA results was evaluated by comparing with the PFAS concentration and fluorine content in the present repellents determined by the other methods. Although DMA fairly represented the concentration of impure PFAS in the samples, PMA led to overestimation of the emission potential. We suggest use of single-shot injection and the standard addition method could improve the PFAS quantification by PMA.

Acknowledgments:
This research was supported by the Environment Research and Technology Development Fund (JPMERF20213002) of ERCA provided by the Ministry of the Environment of the government of Japan.

References:
Chlorinated paraffins (CPs) are flame retardants and plasticizers applied in the manufacture of products such as polyvinyl chloride and rubber, which have been widely documented to migrate from consumer goods within indoor environments and bind with indoor dust, leading to human exposure via inadvertent dust ingestion. Due to evidence of bioaccumulation in humans and toxic properties, restriction on the manufacture of some CP classes has been enacted in many countries during the past two decades. Recent reports have suggested that CP mixtures substituted with bromine, i.e. bromochloro alkanes (BCAs), may be utilized as CP replacements [1] and have similar potential to contaminate indoor environments [2]. Given the physicochemical similarities between CPs and BCAs, comparable contamination and health risks may be expected of the emerging BCAs, though very little data is currently available.

Materials and Methods: Four analytical mixture standards of C14-BCAs with varying halogenation degrees were synthesized and used for verification of extraction, analysis and identification methods previously applied for CP analysis [3-4]. Indoor dust samples from Australia (n=10), Belgium (n=10), Colombia (n=10), Japan (n=10), Thailand (n=10) and United States of America (USA, n=10) were analysed by both GC-ECNI-Orbitrap-HRMS and LC-Orbitrap-HRMS. A custom-built “CP-Seeker” software was used to seek and integrate BCA homologues ranging from C6-36, Br0-12 and Cl0-30 (Br+Cl≥3) based on detection criteria of isotopic pattern matching >80% and accurate mass deviation <2 mDa with respect to theoretical values.

Results: C14-BCA homologues ranging Br1-7 and Cl2-8 were observed among the four synthesized standards and good sample extraction efficiency was indicated by spike and recovery tests. BCAs were detected in seven out of the 60 indoor dust samples analysed, each from the USA. Among the samples, BCA homologues of C-chains C8, C10, C12, C14, C16, C18 were each identified with overall halogenation ranging Br1-7 and Cl3-8. C18-BCAs were the most prevalent among the samples, followed by C12-BCAs. BCAs of Br1 and Br2 were dominant among samples while homologues of Br3 and Br4 were detected with lower frequency.

Discussion and Conclusion: To the authors knowledge, the only other report of BCAs measured in the environment describes contamination of indoor dust, air and sewage sludge from Australia [2]. He et al., [2] determined similar detection rates of BCAs in Australian indoor dust to those observed within the USA samples of the present study, which may suggest that these emerging contaminants have only been used in specific global regions. The distribution of Br and Cl among homologues was also approximately similar between the Australian and USA samples, while the prevalence of even numbered C-chain lengths observed in the USA was not apparent in the Australian indoor dust analyses. While no commercial or industrial standards are available for the quantification of BCA levels measured in indoor dust, the successful synthesis of BCA mixtures with defined C-chain length provides a strong basis for the development of future quantitative protocols.

References:
Introduction: Waste electrical and electronic equipment (WEEE) represent a vast and burgeoning reservoir of a diverse array of both legacy and "emerging" organic pollutants. These chemicals have been primarily added at weight percent levels to electrical and electronic equipment (EEE) plastics used in casings, wiring, and printed circuit boards as flame retardants (FRs) and plasticizers. One class of such chemicals is per- and polyfluoroalkyl substances (PFAS), for which little is known about their presence in e-waste recycling facilities, especially in North America. With the new impetus to localize chips production back in the U.S., it is crucial to better characterize this additional possible source of PFAS to the environment and for people.

Materials and Methods: In this study we investigated 82 legacy and novel PFAS in indoor dust from Canadian e-waste dismantling facilities (n=7). Samples were analyzed using LC/MS/MS and GC/MS following previously reported procedures. For the direct total oxidizable precursor (dTOP) assays, samples were treated with a freshly prepared oxidation solution (200 mM K2S2O8, 500 mM NaOH in water) and incubated at 85–90 °C for 7 h before being cleaned up on an Oasis WAX cartridge. For hydrolysis, samples were treated with 1 M NaOH solution in methanol/water (90:10) and incubated at 60 °C for 16 h, followed by liquid-liquid extraction with methyl tert-butyl ether/n-hexane (1:1).2

Results: PFAS were detected in all samples in the range of 285 – 3360 ng/g with a median concentration of 512 ng/g. The profiles varied significantly among samples, although PAPs were generally the most abundant, representing 32 to 63% of PFAS concentrations. 8:2 FTMAC and 6:2 FTS were also abundant in some samples.

After hydrolysis, the concentrations of 3 fluorotelomer alcohols (6:2 FTOH, 8:2 FTOH and 10:2FTOH) increased on average ~100 times, that of 2 perfluorooctane sulfonamido ethanol (FOSEs -MeFOSE and EtFOSE) by 15 and 10 times, respectively. After dTOP assay, the levels of perfluorooalkyl carboxylic acids (PFCAs) increased up to 280 times. Estimates of total daily intake of perfluorooalkyl acids (PFAA) from e-waste dust at low-, intermediate-, and high-risk scenarios were 7.23, 31.8, 604 pg/kg/day, respectively.

Discussion: These are the first data showing the presence of PFAS in North America. For comparison, the levels of polybrominated diphenyl ethers (PBDEs) and novel brominated flame retardants in samples from the same location were in the range of 24,000 to 270,000 ng/g and 12,000 to 420,000 ng/g, respectively. The hydrolysis and dTOP assays revealed the presence of a large amount of PFAAs precursors (e.g. fluoropolymers) in dust samples. The high abundance of FTOH and PAPs is of concern as these compounds were shown to be toxic and can transform into persistent and toxic end-products, PFAAs. Although the daily intake was calculated for the sum of all PFAS, the estimates for the worst-case scenario are higher than the US EPA draft reference dose for PFOA and PFOS (1.5 pg/kg body weight/day and 7.9 pg/kg body weight/day, respectively).

Conclusion: Even though PFAS concentrations in these samples were significantly lower than those of other toxic additive chemicals like flame retardants, it should be noted that the targeted analysis captures only a small fraction of the totality of PFAS present, as shown by the hydrolysis and dTOP assay. Hence, the presence of PFAS in dust from WEEE dismantling facilities is a serious concern both for workers occupational exposure and for the risks associated with the environmental release. More studies are needed to better understand this route of exposure.

Acknowledgments: The authors thank Miriam Diamon and Erick Reiner for kindly providing the samples.

References:
Environmental and Human Exposure to Plasticizers and Other Consumer Product Chemicals
G.Poma & C.Pirard

TUE-PM1-B3  High-throughput effect-directed analysis strategy to study thyroid hormone disruptors in WEEE contaminated toy materials

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2Research Centre for Experimental Marine Biology and Biotechnology (PIE), University of the Basque Country (UPV/EHU), Plentzia, Basque Country, Spain
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Introduction: Plastic products often contain chemical additives to improve and/or modify a specific property of the product. However, these additives are also known to be harmful to human health1,2. Although regulation on certain additives (e.g. polybrominated diphenyl ethers, phthalates, bisphenol-A, and metals) exists within the EU, plastic products containing these additives still enter the European market as a result of poorly controlled recycling practices or none existing regulation in countries where the products are produced. The REACH Directive3 restricted the content of some phthalates and PBDEs to 0.1 % in toys considering their endocrine disrupting potential4. Thyroid hormones play a key role in the development and maintenance of a normal physiological state5 and a deficiency of such hormones can cause neurodevelopmental effects in both rodents6 and humans7. In this thread, Brandsma et al.8 observed high migration rates of certain additives present in some commercially available toys by simulating the mouthing behavior of toddlers. The aim of this study is to elucidate whether chemical mixtures of those polymeric toy materials and their saliva leachates operate as thyroid hormone disruptors, and to adopt a high-throughput effect directed analysis (EDA) strategy to identify specific drivers.

Materials and methods: Toys commercially available in the EU market were pre-selected depending on availability and presence of contaminants8. The saliva leaching experiment was performed as described by Ionas et al.9. Briefly, toy samples (10 cm²) were leached using artificial saliva for 1 h. The clean supernatant was submitted to both solid-phase and liquid-liquid extractions. In parallel, a solid-liquid extraction (Plastic SLE) of the toy samples using Acetone:Hexane (50:50) was performed. Obtained extracts were divided and processed accordingly for assessment of the thyroid hormone disruption using the TTR-binding assay10. Samples showing the higher TTR-binding effects were subjected to high-throughput EDA11. Extracts were fractionated by HPLC coupled to a FractioMate™). Suspect and nontarget screening of the extracts was performed using a qTOF (Bruker).

Results and Discussion: TTR-binding experiments showed a concentration dependent activity in all analysed extracts. TTR activity was scored in all samples from highest to lowest. Components extracted directly from the plastic toys showed the highest potential for thyroid hormone disruption. Moreover, saliva leaching extracts from one particular toy appeared to have a non-negligible TTR-binding activity when compared to the other toys. Extracts with the highest activity were selected for further fractionation: SLE for sword, excavator 1 and gun toys, and all sword-saliva leaching extracts.

Two TTR-binding active fractions (fractions 50 and 51) stood out in all the fractionated Plastic SLE (Figure 1) considering an IC50 threshold. The alignment of the bioassay results with the corresponding MS-chromatogram and subsequent feature identification will be presented. Our current results indicate that the activity drivers might be the same in all samples. Moreover, the saliva leaching extracts of the Sword were also fractionated and evaluated, showing some minor active fractions.

Conclusion: This study intended to assess the mixture thyroid hormone disruptive activity of toy chemical additives and their saliva leachates, and unravel the potential activity drivers using a high throughput EDA. Key activity drivers are more isolated using such approach, therefore cause-effect relations can be directly explored.

References:
1. Aurisano et al., 2021. Environ. Int., 146, 106194.;
3. Regulation (EC) No 1907/2006 - (REACH); 2006.;
The main objective of the current study is the assessment of the daily exposure to OPEs through inhalation during the daily life. To achieve this objective, samples of different microenvironments from our daily life were collected and analysed to get a global daily exposure according to the average time spent in each microenvironment. Finally, a risk assessment study will be done to see if the concentrations levels are or not over the risk threshold.

2. Materials and Methods:
PM2.5 samples were collected in quartz microfiber filters using a personal environmental monitor coupled to a Leland Legacy Pump at a flow rate of 10 L/min. With this equipment, a sampling campaign was conducted in indoor environments from Barcelona (Spain) and surrounding cities. Also, urban background samples were collected to compare them with the indoor environments.

A total of 56 microenvironments were sampled, including different rooms from houses (bathroom, bedroom, kitchen and living room), means of transport as car or public transport (buses, trams and metro) and workplaces. A wide variety of workplaces were included such as workshops (computer, electronic waste (e-waste) dismantling, motorbike, painting, pottery and sewing), hairdresser, office, laboratory, and a warehouse. Sampling duration varied from 6 to 12 hours, depending on the microenvironment, being lower for the means of transport samples.

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Filter samples were placed in a 40 mL glass-centrifuge tube and spiked with 15 µL of the OPEs internal standard (IS) solution (d15-TDCIPP, d27-TNBP, d12-TCEP, d15-TPHP, d15-TEP, d21-TPP and d15-TEHP) at 1 ng/µL and let equilibrate overnight. Extraction was carried out with ultrasound, adding 10 mL of hexane:acetone (1:1). The extraction was performed twice for each sample, both extracts were combined and filtered with glass wool. Filtered extracts were evaporated using a gentle nitrogen stream up to a volume around 2 mL and then transferred to a 2 mL chromatography vial. Extract was finally evaporated to incipient dryness and immediately reconstituted with 500 µL of methanol.

Samples were subjected to an online sample purification and analysis with a Thermo Scientific TurboFlow™ system consisting of a triple quadrupole (QqQ) MS with a heated-electrospray ionisation source (H-ESI), two LC quaternary pumps and three LC columns, two for purification and one for separation. The TurboFlow™ purification columns employed were Cyclone™-P (0.5x50mm) and C18-XL (0.5 × 50mm). Chromatographic separation was subsequently achieved using an analytical column: Purosphere Star RP-18 (125mm×0.2mm) with a particle size of 5 µm (Giulivo et al., 2016). Detailed conditions used for purification and chromatographic separation, as well as the selective reaction monitoring transitions used for the quantification and confirmation, can be found in the paper from Balasch et al. (2022).

3. Results:
A total of 15 out of the 20 analysed OPEs were detected in PM2.5 samples, where tripropyl phosphate (TPrP), bis(4-isopropylphenyl) phenyl phosphate (B4iPPP), tris(2-isopropylphenyl) phosphate (IPPP) and tris(2-ethylhexyl) phosphate(TEHP) were the ones not detected in any PM2.5 sample. Number of samples from each group, concentration range, mean and median are summarized in Table 1. Concentration levels of ∑OPEs ranged between 4.37 and 75.4 for houses, 26.3 and 185 in the means of transport and 5.48 and 129 for the workplaces. Most abundant OPEs were similar in almost all the samples, where TCIPP was the most abundant in all the samples, followed by the triethyl phosphate (TEP) and tri-n-butyl phosphate (TNBP). In the car samples there was also a notable dominance from the tris(1,3-dichloro-2-propyl) phosphate (TDCIPP), where it had higher concentrations than in the rest of the samples.

Table 1: Sample information including the sampled locations, the number of samples, the time spent in each microenvironment, and concentration and EDI information (range, mean and median).

<table>
<thead>
<tr>
<th>Microenvironment</th>
<th>N Samples</th>
<th>Exposure duration (h)</th>
<th>Concentration (ng/m³)</th>
<th>EDI (ng/(kg bw×day))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Range</td>
<td>Mean</td>
</tr>
<tr>
<td>House rooms</td>
<td>40</td>
<td>14.5</td>
<td>4.37-75.4</td>
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</tr>
<tr>
<td>Cars</td>
<td>3</td>
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<td>42.9-75.5</td>
<td>58.4</td>
</tr>
<tr>
<td>Public transports</td>
<td>5</td>
<td>0.62</td>
<td>26.3-185</td>
<td>74.1</td>
</tr>
<tr>
<td>Workplaces</td>
<td>17</td>
<td>6 or 8</td>
<td>5.48-129</td>
<td>45.8</td>
</tr>
<tr>
<td>Urban background</td>
<td>2</td>
<td>0.88</td>
<td>11.2-37.2</td>
<td>24.2</td>
</tr>
</tbody>
</table>

EDI through inhalation was calculated from the concentration levels of the different OPEs. To do this, the concentration (ng/m³) of each compound was multiplied by the respiration (19.92 m³/day) to normalize the exposure duration and the body weight, which was considered 70 kg. With these calculations the EDI value is obtained (Table 1), which represents the daily nanograms inhaled for kilogram of body weight: ng/(kg bw×day). This calculation assumes the total absorption in the airways of the inhaled chemicals. The EDI values ranged between 0.10 and 12.2 ng/(kg bw×day) (median 0.59 ng/(kg bw×day)), finding the microenvironments with a higher exposure those from working, probably due to the elevated amount of time that is spent at work, which was considered 8 h. Even so, the exposure duration in the rooms from houses was longer, but the EDI lower, which indicates that the higher concentrations from the rooms correspond to those where less time is spent daily, decreasing the EDI value. The median total EDI that would correspond to a complete day would be of 4.74 or 4.59 ng/(kg bw×day), considering car or public transport as mean of transport, together with the median values from rooms, workplaces, and urban background for both cases.
For the calculation of the EDI value, only the quantified OPEs with oral slope factor (SFO) and oral reference dose (RfD) reported by USEPA (2019) were considered. This results into the reduction of the OPEs that are used for the risk assessment, decreasing from up to 20 to only 10 OPEs, which are TEP, tris(2-chloroethyl) phosphate (TCEP), triphenylphosphine oxide (TPPO), TCIPP, TDCIPP, triphenyl phosphate (TPHP), TNBP, tricresyl phosphate (TMCP), 2-ethylhexyl diphenyl phosphate (EHDP) and TEHP. The reduction must be done because only some compounds have established values of oral slope factor (SFO), which is related with the carcinogenic risk (CR), and the oral reference dose (RfD), which is related with non-carcinogenic risk (non-CR).

To calculate the CR, the EDI of the compound is multiplied by their SFO. The risk threshold is considered if there is one cancer incidence case per million people, meaning that if the CR exceeds 1×10⁻⁶, it will be considered a risk situation. CR was calculated for each of the environments, using the mean value that represents the general situation, and the worst possible situation by picking the microenvironments with higher risk. In both situations public transport was selected as the mean of transport, as all values were similar to the ones from car. The median value for CR through inhalation was 6.82×10⁻⁹, which is 150 times lower than the risk threshold. In the worst situation, the CR value was 1.02×10⁻⁷, which is 1500 times below the risk threshold and can be increased by other sources of human exposure. Main contribution to the median CR risk comes from the workplace, followed by the bedroom (Figure 1). Both microenvironments are the ones where most time is spent daily, which is coherent with the higher contribution to the total risk. In the worst case situation, public transport was selected due to the similarity in the median value and the huge difference in the maximum one. For the CR risk, the median value was 6.82×10⁻⁹, which is 150 times lower than the risk threshold, and considering the worst situation the risk was 1.02×10⁻⁷, which was 9 times below the risk threshold and can be increased by other sources of human exposure. For the non-CR risk, the median value was 5.98×10⁻⁴, being 1670 times lower than the risk threshold, and considering the worst situation the risk was 1.91×10⁻³, being 522 times lower than the risk threshold. Also, for the non-CR risk the main contribution came from the workplace in the median and maximum situations, but in this case the workplace that represented an elevated non-CR was the X, contributing to a 54% to the sum of all non-CR (Figure 1).
4. Discussion:
Comparing the results from the present study with other ones, it can be found that the levels from the studied microenvironments are in general similar or inferior to those previously reported from similar environments in other cities. In the case of the indoor environments from Hu et al. (2019), reported levels are higher, going up to 306 ng/m³, while the highest concentration reported in the present study was slightly superior to the half of that, 185 ng/m³. When levels are compared with those corresponding to houses and offices from Australia (median 44 ng/m³) (He et al., 2018), levels would be certainly similar to median value from Barcelona’s workplaces, but clearly superior to the one corresponding to houses was below this value (36.9 and 12.2 ng/m³, respectively). In the case of the study from Norway, the mean concentration in the residential living rooms was 99.2 ng/m³, which is much higher than the one reported in the present study for houses. For the school classrooms, the concentration was 41.9 ng/m³, being slightly superior to the one from the workplaces of this study, but a smaller difference compared with the houses. Also, in both studies (Australia and Norway), the most abundant compound in all the samples was the TCIPP, being the same than in the current study.

Moving to the EDI values, the levels reported from Hu et al. (2019) are slightly higher, as in the present study the median values were 4.74 and 4.59 ng/(kg bw×day), while in their case they reported mean EDI for indoor was 9.2 ng/(kg bw×day), almost doubling the value from this study. EDIs that were reported in the studies from Australia and Norway are closer to the one from the present study, but in both cases the value is slightly higher (7.9 and 5.8 ng/(kg bw×day), respectively) (Cequier et al., 2014; He et al., 2018). Even if the rest of values are higher, none of the other studies reported risk for the concentrations of exposure, which agrees with the results from the present study.

Comparing the levels of inhalation with those from ingestion, it can be seen that the contribution from inhalation is low, as the EDI that corresponds to inhalation based on the UK diet is 35.8 ng/(kg bw×day) (Gbadamosi et al., 2022). When this value is compared with the one obtained in this study for the inhalation exposure pathway, it can be seen that it is 7 times higher. Also, dermal contact exposure via contact with indoor dust is similar than the one that corresponds to inhalation, for example Abou-Elwafa Abdallah et al. (2016) reported a median EDI value of 4.1 ng/(kg bw×day) for some OPEs, which would be pretty similar to the inhalation EDI. Considering these values, we could say that the EDI of OPEs is divided into an 81% for the ingest, a 10% for the inhalation and a 9% for the dermal contact pathways.

5. Conclusions:
Concentration levels of 16 OPEs were determined in PM2.5 samples from different microenvironments from our daily life, with concentration levels ranging between 4.37 and 185 ng/m³. Highest concentration of OPEs were those from the bus (public transport), a laboratory (workplace) and the e-waste dismantling (workplace). The OPEs detected in higher concentrations were TCIPP, TEP, TCEP and TNBP, all of them reported to present non-CR.

A risk assessment through inhalation has been performed for those samples, EDIs were calculated and found to range between 0.10 and 12.2 ng/(kg bw×day), and from these values it was determined the non-CR and the CR (6.07×10⁻⁴ and 6.66×10⁻⁹, respectively), finding that in none of the possible situations the risk levels were superior to the risk threshold. Even so, the levels in the worst situation, which was calculated from the combination of the environments that presented the highest risk, are close to the risk threshold for CR, and might be outreach if other exposition sources as ingestion or dermal contact are considered.

The next step will be the comprehensive analysis of the daily intake, considering all three sources of exposure: inhalation, ingestion, and dermal contact, which will result into a complete risk assessment of the human exposure to OPEs.

6. Acknowledgments:
This study was supported by the Spanish Ministry of Science and Innovation (Project EXPOPLAS PID 2019-110576RB-I00, and RTI2018-098095-B-C21), by the Severo Ochoa Project CEX 2018-000794-S funded by MCIN/AEI/10.13039/501100011033 and by the Generalitat de Catalunya (Consolidated Research Group 2021SGR01150). A. Balasch thanks his fellowship PRE 2020-091979. The authors would like to thank the volunteers that helped during the sampling campaign and the workers that accepted sampling the workplaces.
TUE-PM1-B4  Assessment of inhalation exposure to plasticizers and flame retardants in indoor environments

7. References
Environmental and Human Exposure to Plasticizers and Other Consumer Product Chemicals

G. Poma & C. Pirard

1. Introduction:
Facemasks are a common product of personal protection equipment (PPE) in a variety of industries, including healthcare, manufacturing, and construction. Disposable masks are made of polymers such as polypropylene, polyurethane, polycrylonitrile, polystyrene, polycarbonate, polyethylene, or polyester (Potluri & Needham, 2005). Many additives, including plasticizers, antioxidants, and flame retardants, are added to these materials in non-covalent forms to obtain products with greater performance; they are commonly used to increase their flexibility and durability. Plastic additives, such as organophosphates esters (OPEs) (Fernández-Arribas et al., 2021) and phthalates (Cao et al., 2023; Xie et al., 2022), have been detected in different types of facemasks with a level up to 27.7 µg/mask and 37.4 µg/g respectively. These chemicals can be released from the plastic material and inhaled by the wearer, leading to potential health hazards. However, the possible health dangers linked to breathing in plasticizers and other chemical substances included in facemasks, have not been fully researched. Facemasks that are commonly used for an extended period of time have raised concerns about the potential negative effects on human health. Exposure to plasticizers has been linked to respiratory diseases, endocrine disorders, and reproductive problems. For instance, di (2-ethylhexyl) phthalate (DEHP) has been linked to insulin resistance, overweight, and obesity, as well as an increase in allergy disorders and asthma in children (Benjamin et al., 2015; Trasande et al., 2013). In addition, other chemical compounds, such as tri-n-butyl phosphate (TNBP) have been shown to interfere with endocrine and reproductive processes as well as, nervous system development (He et al., 2020). Additionally, a few OPEs and phthalates have defined oral reference doses (RfD) and oral cancer slope factors (SFO), which were updated by the USEPA (2019).

The aim of this study is to assess the additional exposure to plasticizers and other chemical compounds associated with plastic through inhalation, suffered by people who regularly wear facemasks as PPE. It is important to note that the findings of this study will have significant implications for public health and safety. Facemasks are a necessary piece of PPE in many industries, and the COVID-19 epidemic has recently increased the need for facemasks. The safety of people who wear facemasks as PPE will be improved by the identification of possible health concerns linked with their usage.

Firstly, this study will be conducted by collecting facemasks worn by individuals in various industries and during COVID-19 and will analyse the occurrence of different groups of plastic additives in them, such as OPEs, phthalates, and some new alternative plasticizers. Additionally, the study will assess the level of exposure to these chemicals by measuring the concentration of plasticizers and other chemical compounds in the air breathed by the wearer. The results of this study will be used to evaluate the potential health risks associated with the use of facemasks.

2. Materials and Methods:
The experiment involved the use of over 57 different types of facemasks to assess the presence of plastic-associated chemicals. Out of the total number of facemasks analysed, 35 were various brands of self-filtering masks, including four KN-95, 11 FFP2, and 20 FFP3 masks. And 11 of both surgical masks and reusable masks were also included in the analysis. The packaging information revealed that most brands utilized synthetic materials such as non-woven P, PE, PET, recycled PET, cotton, and nylon. Several units of each type of facemask have been purchased in order to carry out the different analyses. The presence of a total of sixteen OPEs, sixteen phthalates, and four alternative plasticizers (two adipates, a citrate, and 1,2-cyclohexane dicarboxylic acid diisononyl ester (DINCH)) were studied in the selected facemask.

To investigate the release of chemical additives present in facemasks during their prolonged use, an inhalation study was conducted. Paper-mâché dummy heads, measuring 20 cm in height, 15 cm in width, and 20 cm in depth, were employed to simulate human heads. Airborne particulate matter was collected using a PM2.5 head connected to a 37 mm quartz microfiber filter, and this setup was linked to a Personal Environmental Monitor (PEM) via an anti-electrostatic inlet tube. Each experiment ran for 10 hours at a flow rate of 10 L/min, reflecting the volume of air typically inhaled by an adult for 8 hours, which aligns with the recommended duration of facemask use.

The experiments were conducted under three different conditions to explore the influence of humidity and temperature on the release of plastic chemicals from facemasks. These conditions included the normal conditions of a Mediterranean city like Barcelona during autumn and winter (22°C and 50% humidity), high temperatures (35°C and 50% humidity), and high humidity (22°C and 80% humidity). To enhance experimental conditions and better simulate human respiration, additional tests using thermal control mannequins were also performed.
Extraction methodologies previously established were employed for both the facemasks and quartz filters. Before extraction, components such as ear loops, metal nose strips, valves, and adhesive sticks were removed from the masks. The masks were then weighed and cut into small pieces (1-2 cm²) before being placed in glass beakers. The quartz filters were cut and transferred into 40 mL glass-centrifuge tubes. All samples were fortified with a 25-ng internal standard mixture. (d_{15}-TEP, d_{17}-TDCIPP, d_{17}-TNBP, d_{17}-TCEP, d_{17}-TPHP, d_{17}-TEHP, d_{15}-DMP, d_{15}-DEP, d_{12}-DCHP, d_{15}-DBP, d_{15}-DEHP, d_{15}-DHexP, d_{15}-BBzP, and d_{3}-ATBC). Ultrasound extraction was performed for 15 minutes using 15 mL of hexane:acetone (1:1) for filters and 60 mL for mask samples. The extraction process was conducted twice, with both extracts combined and filtered using glass wool. The solvent was subsequently concentrated to near dryness and reconstituted with methanol to a final volume of 500 µL. Online sample purification and analysis of the samples were performed using a Thermo Scientific TurboFlow™ system coupled to a triple quadrupole (QqQ) MS. Two TurboFlow™ purification columns, Cyclone™-P (0.5x50mm) and C18-XL (0.5x50mm) were used for the purification process. Then, using an analytical column, Purosphere Star RP-18 (125mm 0.2mm) with a particle size of 5 m, chromatographic separation was accomplished (Giulivo et al., 2016).

Detailed conditions used for purification and chromatographic separation, as well as the selective reaction monitoring transition used for the quantification and confirmation, can be found in the paper from Fernandez-Arribas et al. (2021). The extraction method for both matrices was validated by calculating parameters such as recoveries, reproducibility, limits of detection (LODs), and limits of quantification (LOQs), as well as the reproducibility of the inhalation experiment was assessed.

3. Results:
The concentrations of plastic additives in the facemasks are summarized in Table 1. OPEs and phthalates were detected in all the analyzed samples, albeit at varying concentrations spanning a wide range. OPE concentrations ranged from 0.09 to 57.1 µg/mask, while phthalate concentrations ranged from 0.002 to 742 µg/mask. Among the alternative plasticizers, DINCH was found in only four of the analyzed samples, indicating its lower occurrence. Adipates, although less frequently detected, were present in higher concentrations ranging from 0.09 to 238 µg/mask. Citrates were the most frequently found alternative plasticizers, although in lower concentrations.

Table 1: Plasticizer levels in mask samples expressed in µg/mask (mean(max-min)).
Analysing all 40 samples, it was observed that FFP3 masks exhibited the highest levels of plasticizers, with a mean concentration of 616 µg/mask. In contrast, KN-95 and surgical facemasks showed lower values of chemical additives, with concentrations ranging from 0.76 to 21.3 µg/mask.

Regarding the inhalation study, the developed method showed good reproducibility, as evidenced by the low RSD values (from 3 replicates), all below 9%, for all the compounds analyzed (Table 2). To assess the influence of temperature and humidity on the release of these chemical additives, we performed the experiments under three different conditions. The results presented in Table 2 provide an illustrative example of three compounds detected in the PM2.5 filters following the experiments done with an FFP2 mask. TEP and TCIPP were specifically released when higher temperatures were applied, whereas TNBP showed an increased release at elevated temperatures. Interestingly, we noted that high humidity conditions had a minimal impact on the release of compounds.

Table 2: Plasticizer levels found in 2.5PM filters after the 8 hours of inhalation experiments with FFP2 mask type (expressed in ng release during 8h of use).

<table>
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<th>TEP</th>
<th>TCIPP</th>
<th>TNBP</th>
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<td>Initial conditions (22ºC and 50% humidity)</td>
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<td></td>
<td>RSD%</td>
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<td>High temperature (35ºC and 50% humidity)</td>
<td>Average</td>
<td>50.8</td>
<td>7.09</td>
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<tr>
<td></td>
<td>RSD%</td>
<td>9</td>
<td>4</td>
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<tr>
<td>High humidity (22ºC and 80% humidity)</td>
<td>Average</td>
<td>28.4</td>
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<td>RSD%</td>
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By utilizing the measured values of chemical additives found in PM2.5 filters obtained after an 8-hour simulation of mask usage, along with the initial levels of plasticizers found in the studied masks (refer to Table 1), the estimated percentage of each compound that would be inhaled during mask use was determined. These experiments were conducted under high-temperature conditions, as it was found to be the most unfavourable scenario, resulting in increased release patterns of the compounds. Moreover, these elevated temperature conditions align with the most representative circumstances for investigating human inhalation, given that human respiration typically occurs within the range of 33-34ºC.

Out of the 36 compounds examined, only 15 were detected in the PM2.5 filters, representing a subset of 5 OPEs, 6 phthalates, and 3 alternative plasticizers. Among these, the phthalate compounds exhibited the highest inhalation percentages, with DEHP being the most commonly released during facemask usage (42% of release). Conversely, alternative plasticizers were less frequently detected in the PM2.5 filters, with ATBC being the most prevalent among them. Specifically focusing on the OPEs, our findings revealed that TEP had the highest inhalation percentages, ranging from 8 to 19%, followed by TNBP with percentages ranging from 0.3 to 7%.

4. Discussion:
Some investigations have been conducted to explore the occurrence of plasticizers in facemasks, providing valuable insights into the presence and concentrations of these chemical compounds across different mask types. Fernandez-Arribas et al. (2021) reported a range of 0.02 to 27.7 µg/mask for organophosphate esters (OPEs), with KN-95 masks (11.6 µg/mask) and FFP3 masks (14.1 µg/mask) exhibiting the highest OPE values. The current study aligns with similar concentration ranges (mean values ranging from 0.23 to 14.6 µg/mask), with FFP3 masks consistently demonstrating higher OPE concentrations (mean value of 14.6 µg/mask). The predominant OPE compounds identified in both studies are TEP and TPHP. In the analysis of phthalates, Cao et al. (2023) reported median values of 1.24 µg/g, slightly lower than the median values of 2.05 µg/g observed in this study (note that our concentration levels expressed in µg/mask were transformed in µg/g for the comparison). This difference can be attributed to the larger number of phthalates analyzed and the greater sample variety in our study. However, both studies identify DNBP and DEHP as the predominant plasticizers. Importantly, these results are consistent with findings from studies conducted in other five countries: Europe (2.89 µg/g), China (2.05 µg/g), USA (1.95 µg/g), Japan (1.46 µg/g), and South Korea (0.79 µg/g) (Xie et al., 2022). FFP3 masks consistently demonstrate high concentrations of plasticizers in most of the reviewed studies. It is crucial to consider the variations in mask weights across different types, with FFP3 masks weighing approximately 23 g and surgical masks weighing around 1.8 g. Therefore, expressing concentrations per gram of sample provides a more uniform approach. For instance, when comparing the concentration levels of surgical masks (which exhibit lower concentration levels) and FFP3 masks (which display higher concentration levels), the concentration expressed in µg/g of facemask presents higher congruence. Specifically, surgical masks demonstrate a concentration range of 11.5 µg/g, while FFP3 masks exhibit a concentration range of 33.3 µg/g. Regarding the inhalation experiments, the levels of released compounds in this study were generally higher than those reported in the previously published study (Fernandez-Arribas et al., 2021), likely due to the high-temperature conditions used in our experiments, which proved to be the scenario with the highest concentration of released compounds. The OPEs released from facemasks during an 8-hour period in our study ranged up to 19%, while Fernandez-Arribas et al. (2021) reported levels of around 10% for all compounds.

5. Conclusions:
Overall, results demonstrate that plastic additives, including OPEs, phthalates, and alternative plasticizers, are consistently present in the analyzed facemasks. The concentrations vary widely, with median concentrations of 0.81 µg/mask, 7.37 µg/mask, and 12.3 µg/mask for OPEs, phthalates, and alternative plasticizers respectively. Among different types of facemasks, FFP3 masks exhibit the highest levels while KN-95 and surgical masks demonstrate comparatively lower concentrations. This highlights the variability in plasticizer concentrations across various facemask types. Regarding the inhalation experiments, our findings indicate that only a small percentage of the substances in the facemasks would be breathed during routine use. Additionally, the experiments revealed temperature-dependent release patterns, suggesting that higher temperatures could enhance the release of plasticizers from the facemasks, with a mean inhalation percentage of 19%. Moreover, the results demonstrated diverse release patterns not only between different types of facemasks but also within the same group. KN-95 and reusable facemasks exhibited higher percentages of inhaled compounds compared to other types.

Moving forward, our next step is to enhance the inhalation experiments by incorporating thermal control mannequins. These mannequins will allow us to simulate human inhalation more accurately by controlling body temperature, breath temperature, and humidity. Considering that the inhalation experiment results highlighted the influence of temperature and humidity on release patterns, these factors are crucial to consider in our future investigations.

6. Acknowledgments:
This research work was funded by the European Commission NextGenerationEU (Regulation EU 2020/2094), through CSIC’s Global Health Platform (PTI Salud Global), by the Spanish Ministry of Science and Innovation (Project EXPOPLAS, PID2019-110576RB-100), and by the Generalitat de Catalunya (Consolidated Research Group 2021SGR01150). IDAEA-CSIC is a Centre of Excellence Severo Ochoa (Spanish Ministry of Science and Innovation, Project CEX2018-000794-S).
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Environmental and Human Exposure to Plasticizers and Other Consumer Product Chemicals

G.Poma & C.Pirard

TUE-PM1-B5  Assessing Inhalation Exposure to Plasticizers Suffered by Facemask Wearers

7. References:
TUE-PM1-C1  Are municipal waste incinerator emissions associated with polychlorinated dibenzo-
dioxins/furans and polychlorinated biphenyls in the human milk of women living nearby? 
Findings from the Breast milk, Environment, Early-life, and Development (BEED) Study

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Acknowledgments:
This study is funded as part of a PhD studentship by the National Institute for Health Research’s Health Protection Research Unit in Chemical and Radiation Threats and Hazards, a partnership between UK Health Security Agency and Imperial College London.

Introduction:
Municipal waste incinerators (MWI) are the main way that waste is managed in the United Kingdom (UK). The burning of municipal waste produces a range of pollutants, including polychlorinated dibenzo-dioxins/furans (PCDD/Fs) and polychlorinated biphenyls (PCBs), which have toxic effects on human health. Proxies for exposure to PCDD/Fs and PCBs from MWIs, such as residential proximity from an MWI, are often used in epidemiological studies but these proxies may not accurately reflect an individual’s true exposure.

Some older studies reported that human groups exposed to emissions from MWIs had higher levels of PCDD/Fs than unexposed groups (Campo et al., 2019). However, the evidence on this matter has become inconclusive since the implementation of European MWI emission regulations.

This study is the first time that biomonitoring of individuals around MWIs has been performed in the UK. In this study we aimed to explore whether the levels of PCDD/Fs and PCBs in the human milk of mothers living nearby MWIs were associated with proxy measurements for exposure to MWI emissions. This study will help to improve the interpretation of epidemiological studies that have used the same proxies.

Materials and Methods:
As part of the Breast milk, Environment, Early-life, and Development (BEED) study, primiparous women were recruited from within 20 km of English MWIs between 2013 and 2016 and asked to provide human milk samples. For each participant two exposure proxies were used: (i) straight-line distance to nearest MWI (proximity) and (ii) dispersion models for ground-level PM10 concentration from MWI emissions (modelled PM10).

The samples were analysed using capillary gas chromatography coupled with high-resolution mass spectrometry for quantitative levels of 17 PCDD/F and 12 PCB congeners.

Linear regression models were used to examine the association between the levels of log transformed toxic equivalents (TEQWHO2005) and the two exposure proxies for complete cases adjusting for maternal age, maternal BMI, frequency of consumption of high animal fat containing products and a random intercept for MWI location. Principal component analysis (PCA) was used to assess whether the congener percentage TEQ contribution profiles of the samples clustered by either of the exposure proxies.
Human Exposure
A. Soubry & H. Stapleton

TUE-PM1-C1 Are municipal waste incinerator emissions associated with polychlorinated dibenzo-dioxins/furans and polychlorinated biphenyls in the human milk of women living nearby? Findings from the Breast milk, Environment, Early-life, and Development (BEED) Study

Results:
Samples from 190 women were analysed for PCDD/Fs and 148 for PCBs. Overall $\Sigma$TEQ-PCDD/Fs ranged from 0.7 to 16.3 pg/g lipid with a geometric mean of 3.6 (GSD: 1.8), $\Sigma$TEQ-PCBs ranged from 0.4 to 9.3 pg/g lipid with a geometric mean of 2.1 (GSD: 1.7) and $\Sigma$TEQ-PCDD/F + PCBs ranged from 1.7 to 25.1 pg/g lipid with a geometric mean of 5.8 (GSD: 1.6).

There was an average increase of 8.1% (95% CI: 0.9 to 17.4) in human milk $\Sigma$TEQ-PCDD/F + PCB per doubling in modelled PM10 measurements. Proximity to nearest MWI was not associated with human milk $\Sigma$TEQ-PCDD/F + PCB. In the PCA, the congener toxicity profile did not differ for women who had similar exposure proxy measurements.

Discussion and Conclusion:
The human milk $\Sigma$TEQ-PCDD/F + PCB levels of the participants were in line with other European studies. Earlier studies have provided limited evidence regarding the relationship between exposure to incinerator emissions and biomonitoring of dioxin like compounds, but these studies mostly relied on proximity to measure exposure and measured only PCDD/Fs. The relationship between modelled PM10 and human milk $\Sigma$TEQ-PCDD/F + PCBs is consistent with previous findings that this proxy is correlated with PCDD/F and PCB emissions from UK MWIs (Douglas et al., 2017). Residual confounding may have a role in these findings and future studies would benefit from collecting more detailed individual exposure profiles to account for maternal migration and precise dietary consumption of PCDD/Fs and PCBs. The complex interactions of the individual congeners are unlikely to be captured by statistical approaches and may explain the high levels of clustering in the PCA which was not explained by either exposure proxy, something that has been found in other studies that have used the same approach.

References:
Introduction: Transfused blood may be a potential source of exposure to various environmental pollutants and presents a risk to vulnerable patient groups such as premature infants. The aim of this study was to measure blood concentrations of environmental pollutants in Norwegian donors and evaluate the risk of pollutant exposure through blood transfusions.

Materials and Methods:
Blood donors (n=352) were randomly recruited from three Norwegian blood banks: in Bergen, Tromsø and Kirkenes. Mean age (SD) was 43.0 (14.2) years, age range 19-72 years, 51% were women. Analyses of organochlorine pesticides were performed in serum samples by gas chromatography atmospheric pressure ionisation coupled to tandem mass spectrometers (GC-API-MS/MS; Waters, Milford, MA, USA) as described previously (1), while per- and polyfluoroalkyl substances (PFAS) were measured by ultrahigh-pressure liquid chromatography (UHPLC-MS/MS) coupled to a triple-quadrupole mass spectrometer (2). Additionally, lead and mercury were measured in whole blood using inductively coupled plasma mass spectrometry (ICP-MS).

Results:
Pesticides median (maximum) serum concentrations, pg/mL wet weight were: \( p,p' \)-DDE 180 (6504), \( o,p' \)-DDE 0.45 (3.30), \( p,p' \)-DDT 11.5 (404), \( o,p' \)-DDT 3.1 (25), oxychlordane 19.7 (200), hexachlorobenzene (HCB) 155 (404), heptachlor 1.1 (5.4). PFAS median (maximum) serum concentrations, ng/mL were: perfluorooctanoate (PFOA) 1.16 (4.97), perfluorooctane sulfonate (PFOS) 4.91 (32.3), perfluorohexane sulfonate (PFHxS) 0.62 (10.4), perfluorononanoate (PFNA) 0.52 (2.66). The sum of these four PFAS had median 7.45 ng/mL and range 2.14-39.6 ng/mL. Detection rate for these four PFAS, HCB, oxychlordane, \( p,p' \)-DDE, \( p,p' \)-DDT and \( o,p' \)-DDT was 100%, for heptachlor 76%, for oxychlordane 72%, for \( o,p' \)-DDE 53%, for \( p,p' \)-DDT 49%, for \( o,p' \)-DDT 49%, for \( o,p' \)-DDT 24%.

If EFSA 2018 (3) TWIs of 13 ng/kg bw for PFOS and 6 ng/kg bw for PFOA were applied and calculated for only one blood transfusion per week, then 100% of donors had PFOS and PFOS concentrations over these limits. Altogether 56.3% of all blood donors had sum of four PFAS (PFHxS, PFOA, PFOS, PFNA) over the EFSA 2020 limit for maternal blood 6.9 ng/mL (4). Sum of four PFAS, HCB, oxychlordane, \( p,p' \)-DDE, \( p,p' \)-DDT and \( o,p' \)-DDT were significantly positively associated with mercury (median 9.5 (max 129) nmol/L) concentrations.

PFHxS concentrations were significantly positively associated with blood lead (median 0.06 (max 0.59) µmol/L) and mercury. About 18% of donors had lead concentrations and 11% of donors had mercury concentrations over the limits suggested for transfusions in premature infants.

Discussion and Conclusion:
A considerable percentage of blood donors had PFAS concentrations over the suggested limits. In addition, at each study site, there were donors with high pesticides, lead and mercury concentrations. These pollutants have known detrimental health effects. Screening for pollutants in donor blood may be a feasible approach to avoid exposure through blood transfusions to vulnerable groups of patients such as premature infants.

Acknowledgments: The authors are grateful for the support of the Department of Laboratory Medicine, University Hospital of North-Norway, the Department of Medical Biochemistry and Pharmacology, Haukeland University Hospital, Bergen (Norway), and the Northern Norway Regional Health Authority (Helse Nord RHF).

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TUE-PM1-C3  Concentrations of PCB and PCDD/F in pooled serum samples from Swedish children

Irina Gyllenhammar1*, Matilda Näslund1, Ulrika Fridén1, Pernilla Hedvall Kallerman1, Sanna Lignell1, Emma Hallidin Ankarberg1, Marie Aune1.

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Introduction: The European Food Safety Authority (EFSA) stated in their risk assessment of dioxins and dioxin-like PCBs from 2018, that exposure from food leads to an exceedance of the tolerable weekly intake (TWI) for most of the European population. The TWI was based on effects on semen quality and a no observed adverse effect level (NOAEL) of 7 pg TEQ/g serum lipid in 9-year-old boys was established. The aim of the present study was to evaluate serum levels of dioxins and PCBs in children to investigate possible temporal trends and to compare with the EFSA risk assessment.

Materials and Methods: Levels of PCBs (indicator-, mono- and non-ortho PCBs), dioxins and furans (PCDD/F) were analysed in serum using gas chromatography coupled with high resolution mass spectrometry (GC-HRMS). Pooled serum samples from Swedish children at 4, 8 and 12 years of age (n=22) sampled in 2008-2021 from a mother/child cohort (POPUP-study) and from 12-year-old boys and girls (n=16) sampled in 2016-17 within the national dietary survey Riksmaten Adolescents (RMA) were investigated.

Results: Among the PCBs, the mean concentration in pooled serum was highest for CB 153 (28 ng/g serum lipid). Many of the PCDD/F congeners were below the limit of quantification (LOQ) and the mean level (middle bound, MB) of PCDD TEQ (2.6 pg/g serum lipid) was similar to PCDF TEQ (2.4 pg/g serum lipid). Evaluation of time trends in the POPUP children showed that the serum levels, with a few exceptions, have decreased during the period 2008-2021 for PCBs, however, more data points are needed before conclusions about more specific changes in levels can be drawn. Concentrations of total TEQ in serum from 4-year-olds were higher compared to 8- and 12-year-olds. The large number of samples with concentrations below the limit of quantification made the comparison with EFSA’s risk assessment difficult. Nevertheless, our results show that most 8- and 12-year-olds have levels below or around the NOAEL for 9-year-old boys (Figure 1).

Discussion and Conclusion: The results from the present study show that even though exposure assessments indicate a relatively high exceedance of the TWI, the serum levels in Swedish children are below or around the NOAEL of which the TWI is based on. Concentrations in serum from 4-year-olds were higher compared to 8- and 12-year-olds, probably due to more recent breastfeeding and to a higher food consumption (and consequently intake of PCB/PCDD/F) per kg body weight, in comparison with the older children.

Acknowledgments: The Swedish Environmental Protection Agency is acknowledged for financial support. Appreciation is expressed to the participating children and to the nurse and laboratory staff at the Swedish Food Agency.
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Introduction: Persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), organochlorine pesticides (OCPs) and chlorinated paraffins (CPs), are environmental toxicants that accumulate in human tissues, with potential to cause various health effects. Previous studies have shown an association between POP exposure and TNFα expression levels in visceral adipose tissue of obese subjects1. However, the relationship between POP exposure and chronic low-grade gut inflammation, linked to a range of diseases including metabolic syndrome, has not yet been explored2. Therefore, this study aims to investigate the impact of POPs on chronic low-grade gut inflammation using a subset of the Flemish Gut Flora Project cohort3, one of the largest adult population-wide studies on the gut flora, which aims to investigate links between the gut microbiome, health, diet, and lifestyle.

Materials and Methods: POPs, including PCBs, PBDEs and OCPs, were extracted using the protocol outlined by Dirtu et al.4, while the CPs were analyzed following the procedure described by McGrath et al.5. The targeted analysis of POPs was performed using an Agilent 6890 GC coupled to an Agilent 5975 Series Mass Selective Detector (MSD) operated in electron-capture negative ionization. CPs were analyzed using an Agilent 1290 Infinity II LC coupled to an Agilent 6560 QTOF-MS operated in electrospray ionization negative mode.

Results: A total of 440 serum samples were analyzed for the presence of PCBs, PBDEs, and OCPs, while 44 pooled extracts, each consisting of 10 individual samples, were analyzed for CPs. Of the 26 targeted PCBs, PBDEs, OCPs, 12 were detected in more than half of the samples, with CB-138, -153, -170, -180, -183, and -187 as well as hexachlorobenzene (HCB) being present in all samples. Among the PBDEs, BDE-47 was found in 9% of the samples. The most prevalent POPs were the p,p'-DDE, CB-180, and CB-153, with median concentrations of 515 pg/mL, 230 pg/mL, and 224 pg/mL, respectively. The most prevalent OCP, aside from p,p'-DDE, was HCB, with a median serum concentration of 38 pg/mL, while BDE-47 had a median serum concentration of 0.22 pg/mL. Short-, medium-, and long-chain CPs (SCCPs : C10-13, MCCPs: C14-17, and LCCPs: C18-20) were detected in 48%, 68%, and 7% of the samples, respectively. Among them, MCCPs were the most prevalent, with a median concentration of 16.4 ng/mL, followed by SCCPs with 1.2 ng/mL, and LCCPs with 0.98 ng/mL.

Discussion and Conclusion: The present study provides insights into the levels of POPs in serum samples of the Flemish adult population, which are consistent with previous studies conducted within Europe6. Our findings indicate that the Flemish population is still exposed to various POPs through environmental sources, which may have potential health implications. Exposure to POPs has been associated with alterations in gut microbiota composition and increased gut permeability, which could lead to chronic inflammation7. Many studies have provided insight into the causes and consequences of changes in the gut microbiota. Among the multiple factors involved in regulating the microbiome, exogenous factors such as diet and environmental chemicals have been shown to alter the gut microbiome significantly. Although diet substantially contributes to changes in the gut microbiome, environmental chemicals are major contaminants in our food and are often overlooked. Herein, we summarize the current knowledge on major classes of environmental chemicals (bisphenols, phthalates, persistent organic pollutants, heavy metals, and pesticides). These observations underscore the need for further investigations to explore the potential health effects of POP exposure on gut health. It is hypothesized that the concentration of environmental pollutants, including POPs, is correlated with the levels of inflammatory biomarkers. In the second phase of this study, correlation analysis between the concentrations of environmental pollutants and local inflammatory markers, such as fecal calprotectin, will be performed to allow for a comprehensive analysis of the impact of POPs on chronic low-grade gut inflammation.

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TUE-PM1-C5  Integration of Human Biomonitoring and Wastewater-Based Epidemiology: Advancing Understanding of Exposure Trends in the Population

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Abstract:
Understanding exposure trends in the population is essential for effective public health planning, disease surveillance, risk assessment, research, and public awareness. It enables informed resource allocation, early detection of outbreaks, accurate assessment of health risks, advancement of epidemiological studies, and empowers individuals to make informed decisions regarding their well-being. More than two decades ago, we established a human biomonitoring (HBM) program in collaboration between academia and one of Australia’s leading pathology laboratories. In this program, we utilize de-identified surplus pathology samples pooled by age and gender to assess exposure trends in the population. Sampling and pooling campaigns conducted every two years have provided a foundation for assessing temporal trends in population exposure. These campaigns have utilized serum samples (since 2002)1 and urine samples (since 2011)2 for the analysis. These campaigns have the strength of offering good resolution in understanding exposure trends by age and gender. However, their weakness lies in the limited sample numbers, preventing the resolution of spatial and socioeconomic trends. On the one hand, in 2009/10, a wastewater-based epidemiology (WBE) program was implemented for the purpose of initially assessing illicit drug use within the Australian population.3 One of the strengths of this monitoring system is its ability to provide high temporal resolution and high spatial resolution data. However, a limitation of this approach is the lack of gender or age information, which restricts our understanding of which segments of the population are being exposed. A further limitation is the uncertainty regarding the representativeness of chemicals detected in the sewer as an accurate reflection of population exposure. Through the integration and combination of these initially independent programs, we have undertaken a systematic evaluation of exposure trends in the population.4, 5 In this work, we will showcase data that demonstrate the unique and novel insights provided by this combined approach regarding trends in population exposure.

Figure: Comparison of per capita excreted loads of tobacco alkaloids and metabolites between pooled urine samples and wastewater samples.5

References:
TUE-PM1-D1  Towards a broad understanding of chlorinated paraffins response factors via the study of the LC-ESI-HRMS and GC-ECNI-HRMS couplings

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1. Introduction:
Chlorinated paraffins (CPs) are chemicals of emerging concern. Their industrial production began around the 1930s mainly for their properties as plasticizers, lubricants and flame retardants. The production of CPs is done by chlorination of some fractions of paraffins from petroleum refining. This process of synthesis leads to complex mixtures of CPs, named technical mixtures, that are constituted of n-alkanes with various chain lengths and chlorine contents. The regulation classifies CPs according to their chain length, in three main families: short-chain chlorinated paraffins (SCCPs), medium-chain chlorinated paraffins (MCCPs) and long-chain chlorinated paraffins (LCCPs). Among CPs, SCCPs have been classified as persistent organic pollutants (POPs) within the Stockholm Convention since May 2017 due to their harmful effects on the environment and human health. MCCPs are currently under review to be classified on Annex B by the Stockholm Convention. Because CPs are contaminants of emerging concern, evaluations of the exposure of populations to those chemicals are needed. However, currently, there is no routine analytical method that can reliably quantify CPs. One of the main reasons is the lack of CP standards that can represent the very wide range of homologue group found in contaminated samples. In addition, the interlaboratory studies (ILS) set up by the European reference laboratory for persistent organic pollutants (EURL POPs, Freiburg, Germany) highlight a significant variability of the results compared to a given consensus value. Therefore, the main objective presented in this study is a better understanding of the instrumental responses of CP homologue groups, in order to improve their detection and their quantification. Among the large scale of existing analytical techniques, LC-ESI-(HR)MS and GC-ECNI-(HR)MS couplings are the mainly used ones for CP analysis.

In LC-ESI-MS couplings, some studies already highlighted the influence of the choice of the stationary phase (Matsukami et al. 2020) and the choice of the mobile phase (Perkons et al. 2019; Mézière et al. 2020) on the detection of CPs. Matsukami et al. (2020) showed that the choice of a SB-CN column, compared to the classical used C18-like column, thins the chromatographic peaks of homologue groups leading to a higher detection sensitivity. In GC-ECNI-HRMS couplings, the study of certain instrumental parameters such as the source temperature (Tomy et al. 1998; McGrath et al. 2021) or the choice of the chromatographic column (Hammer et al. 2021) have already been published. However, how those parameters (chromatographic separation and instrumental parameters) can influence the response factors (RFs) of CPs, i.e., their quantification, is poorly understood. To respond to this question, a first study focus on the influence of six LC stationary phase chemistries and two mobile phases on the observed homologue group profiles. For the GC-ECNI-HRMS coupling, the source temperature and the GC column polarity were also evaluated and optimized, as well as new parameters such as the inlet and oven temperature, the column length and film thickness. The comparison of optimized LC-ESI-HRMS and GC-ECNI-HRMS coupling is done on samples from ILS organized by the EURL POPs. Then, to better understand the homologue group RFs observed, a study based on quantum mechanics was carried out at the positional isomer scale. Indeed, a recent study showed that the instrumental responses of CP isomers obtained by GC-ECNI-MS and LC-ESI-MS vary depending on the number and position of the chlorine atoms (Fernandes et al. 2022). Therefore, in order to better understand the impact of the chemical structure of CPs on the instrumental responses, the physico-chemical properties of two positional isomers, 1,2,3,4,5,6-hexachloroundecane and 1,2,4,5,8,9-hexachloroundecane, were modelled thanks to quantum mechanical calculations.

2. Materials and Methods:
Samples: For methods development, a CP standard mixture was prepared by combining six technical standards at equal proportions, including two standard SCCP mixtures (51.5% Cl and 63% Cl), two standard MCCP mixtures (42% Cl and 57% Cl), and two standard LCCP mixtures (36% Cl and 49% Cl). For methods comparison, ILS samples were analyzed and included coconut oils samples (CFA to CFE), lard samples (LAA to LAE), pork sausage (PO) and infant milk samples (IFA and IFB). A detailed description of the fortification of the coconut and lard samples can be found in the publication of Kratschmer and Schachtele (2019). For the PO and IFA samples, the natural contamination was assessed. IFB sample was fortified with SCCP and MCCP standard mixtures.
Sample preparation methods: Extraction and purification were achieved according to Mézière et al. (2020). Quantification of ILS samples was performed according to the methods developed by Reth et al. (2005) and McGrath et al. (2020). A mixture of six 13C-labelled CPs, developed by the Chloffin project, were used as internal standards: 13C11Cl6, 13C12Cl6, 13C14Cl6, 13C15Cl6, 13C16Cl6 and 3C21Cl8 congeners. The 13C13Cl6 isomer was used as recovery standard. The data processing method was carried out automatically using in-house CP-Seeker software on the most abundant [M + Cl] - ions for each homologue group detected for LC-ESI-HRMS coupling and on the most abundant [M - Cl] - ions for GC-ECNI-(HR)MS coupling. All the procedure was done in duplicate for each sample.

LC-ESI-HRMS: Six LC columns with various selectivities (Hypersil Gold™ aQ, Cyanopropyl, C30, phenyl-hexyl, PFP and Hypercarb™) and two mobile phases (ACN/H2O and MeOH/H2O) were tested. Dichloromethane was added post-column. Both [M + Cl] - and [M - H] - ions were monitored. Mass spectra were acquired on an Orbitrap mass spectrometer equipped with a heated electrospray (HESI) source used in the negative mode. The source parameters are the ones previously optimized by Mézière et al. (2020). HRMS data were acquired in full scan mode over the m/z range 200–1500. The resolving power was set at 120,000 FWHM at m/z 200. For each column, the sensitivity and the RF of all homologue groups detected were compared by principal component analysis. The data processing method was carried out automatically using CP-Seeker on the most abundant [M + Cl] - ions for each homologue group detected.

Computational method: For the ECNI response modelling, the calculations of vertical attachment energy (VAE), energies of the lowest unoccupied molecular orbital (LUMO) and the bond dissociation energy (BDE) of the C-Cl bond were carried out (Stemmler and Hites 1988). For the ESI response modelling, with Cl - enhanced conditions, the comparison of the standard enthalpy of formation (ΔfH°) of the [M+Cl] - complexes was carried out. Seven diastereoisomers of each positional isomer (1,2,3,4,5,6-hexachloroundecane (isomer A) and 1,2,4,5,8,9-hexachloroundecane (isomer B)) were built thanks to GaussView 6.0.16 software. Then, the five most stable conformers were generated thanks to MOE 2019.01 software based on the stochastic mode and the OPLSAA force field which are adapted to small organic molecules. Next, geometry optimizations of each conformer were performed using the GaussView 6.0.16 software using density functional theory (DFT). The PBE0 density functional was selected coupled to def2-TZVPD as basis set. Geometry optimization were in the ideal gas state at 298.15 K and 101.325 kPa. Frequency analysis revealed no imaginary frequencies which ascertain the lowest energy structure of the optimized geometries. For the experimental study, each positional isomer was injected in GC-ECNI-HRMS and LC-ESI-HRMS, in optimal analytical conditions, in order to correlate the responses with the modelled physico-chemical properties of each congener.

3. Results:
As regards the LC-ESI-HRMS coupling study, the mobile phases comparisons led to the observation of two main ion species: [M + Cl] - and [M - H] - ions. Using ACN, the absolute intensities of the [M + Cl] - ions exceeded that of the [M - H] - ions. Using MeOH in the mobile phase, the absolute intensities of the [M - H] - species was higher for SCCPs, but lower for LCCPs compared to the [M + Cl] - ions. For both mobile phases, a shift of the homologue group profiles to the higher chlorinated CPs was observed when the [M - H] - ions were monitored, with almost no detection of the very low chlorinated CPs under this form. Concerning columns analysis, a general comparison was done thanks to principal component analysis:
Fate, Detection and Analysis of Chlorinated Paraffins
M. Ricci & A. Fernandes

Towards a broad understanding of chlorinated paraffins response factors via the study of the LC-ESI-HRMS and GC-ECNI-HRMS couplings

As regards the GC-ECNI-HRMS coupling study comparing all the parameters listed in the materials and methods part, optimal sensitivity conditions in the detection of CPs have been highlighted. Those optimal conditions include a GC final oven temperature of 340 °C, an ion source temperature of 200 °C and an inlet temperature of 220–240 °C. Using the Optima 5 HT chromatographic column, a better sensitivity was reached compared to the Optima 1 column. The column length and film thickness of 12.5 m and 0.25 µm, respectively, exhibited the best detection sensitivity on the widest range of CPs. Concerning the homologue group profile, parameters that influence the most the RF of CPs were the temperature of the ionization source and the inlet, the film thickness as well as the chemistry of the stationary phase.

Once optimized, a comparison of the both methods was done on ILS samples. For the two couplings, measurements are repeatable with a variability of less than 15% (n=2). The quantification of the ∑SCCPs and the ∑MCCPs is acceptable (|z-score| < 2) for eight samples out of ten and eight samples out of nine, respectively. The results are questionable (|z-score| < 3) for the naturally contaminated pork sausage (PO-1905) and infant milk (IFA) samples. As no consensus values were determined for these naturally contaminated samples, the variability between these two couplings was determined. A variability of 83% and 56% and is observed for the quantification of SCCPs in the pork sausage sample and IFA samples, respectively. For MCCPs, a variability of 53% and 14% is observed for the pork sausage and IFA samples, respectively.

Concerning the computational study, the injection of isomers A and B in GC-ECNI-MS and LC-ESI-HRMS led to a higher instrumental response for the isomer B. Isomer A presents a VAE, a LUMO energy, a BDE and a ΔfH°([M + Cl]-) of -0.46 ± 0.03 eV, -0.69±0.06 eV, 211 ± 11 kJ/mol and -14 ± 3 kcal/mol, respectively. Isomer B presents a VAE, a LUMO energy, a BDE and a ΔfH°([M + Cl]-) of -0.57±0.02 eV, -0.29±0.06 eV, 281±17 kJ/mol and -18±3 kcal/mol, respectively.

Discussion:
Firstly, the comparisons of the studied LC-ESI-HRMS conditions were all done in Cl- enhanced conditions. The observed shift of the homologue group profile towards the most chlorinated CPs, when the [M - H] ions are monitored, is consistent with the results obtained by Huang et al. (2021). Indeed, they observed a higher abundance of the [M - H] species when the chlorination degree increases. According to Schinkel et al. (2018), this is due to the more acidic properties of highly chlorinated CPs. In our study, the use of an ACN/H2O mobile phase mainly reveals the [M + Cl] ions as already shown in the literature (Perkons et al. 2019). Consequently, ACN/H2O mobile phase is interesting in order to limit the ionic species into the ion source leading to a gain in sensitivity. On the other hand, the use of a MeOH/H2O as mobile phase, by monitoring both [M + Cl] and the [M - H] ions, can broaden the observed homologue group profile for a given sample.

Concerning the columns study, in order to better reveal some interactions between CPs and the selected columns (π-π, dipole-dipole), the mobile phase MeOH/H2O was chosen. ACN/H2O was used for quantification purposes.

The comparison of the columns study shows that the Hypercarb™ column and the direct injection condition lead to different homologue group profiles compared to the 5 other columns (Figure 1a).
This phenomenon could be explained by a different mechanism of CP separation. Indeed, in the case of direct injection, all CPs are detected at the same time leading to highly ion interferences into the source. For the Hypercarb™ column, its particularity is its very broad elution profile of CPs (2 minutes for SCCPs (e.g. C_{18}Cl_{7}) to 50 minutes for longer chains (e.g. C_{18}Cl_{7}), which suggests that a more efficient separation of CPs, particularly isomers, occurs. In order to go deeper in the analysis of the five other columns, a second principal component analysis was realized (Figure 1b). It shows how the choice of the LC column could highlight CP homologue groups according to their chain length or chlorine content (data not shown). Indeed, weakly chlorinated homologue groups have a positive correlation according to PC1 whereas the strongly chlorinated homologue groups have a negative correlation according to PC1(data not shown). Also, SCCPs have positive coordinates according to PC2 whereas longer chains have negative coordinates according to PC2 (data not shown). Therefore, the CN column better reveals the highly chlorinated CPs unlike the PFP column. The C30 column better reveals SCCPs compared to the C18-like column. Based on this latter result, the C30 column was selected as the optimized column for the quantification of SCCPs and MCCPs in ILS samples.

Secondly, concerning the GC-ECNI-HRMS optimisation, source temperature was revealed as a main issue because SCCPs were much better detected at lower temperatures than others CPs, while increasing the source temperature better reveals LCCPs. A compromise was found at 200°C for global CP analysis. The optimal temperature of the inlet is found at 220–240 °C in terms of sensitivity. This optimal value is lower than the one set in the majority of studies dealing with the analysis of CPs by GC-MS (280 °C: Krätschmer et al. (2018); (Xu et al. 2019). Concerning the column characteristics, a length and a film thickness of 12.5 m and 0.25 µm are the optimized parameters to enhance the sensitivity. However, a film thickness of 0.1 µm better reveals LCCPs and highly chlorinated CPs, relative to the sum of CPs. This latter result is consistent with those reported by Björklund et al. (2004) for the highly chlorinated PBDEs. Finally, the column chemistry was studied for CP analysis and no retention improvement was observed by using a more polar column (Optima SHT) compared to an apolar column (Optima 1). This observation is not consistent with the one reported by Hammer et al. (2021), where they highlighted a higher retention index of some CP isomers when the column polarity increases.

The comparison of the two optimized couplings described above was done by quantification of ILS samples. According to Kratschmer and Schachtele (2019), ILS showed important variability between laboratories for the analysis of coconut fat and lard samples. Their study highlights that the choice of the instrument, calibration standards and the data processing methods are the parameters that influence the most the quantification of CPs. In our case, only the choice of instruments is different. Therefore, by using the same standards and data processing method, it is possible to homogenize the result for the fortified samples. However, further developments are needed for the naturally contaminated samples. Indeed, the observed homologue group profiles are drastically different between the two couplings, as the GC-ECNI-HRMS method tends to enhance the detection of the highly chlorinated CPs (data not shown), a better quantification would pass on a homogenization of the RFs of the homologue groups.

At the end, one of the ways to homogenize the CPs instrumental responses would be to obtain theoretical RFs based on quantum mechanical calculations. The preliminary results obtained in our study show that the VAE values are negative for the 2 CP isomers. As VAE value is the opposite of the vertical electron affinity (VEA) (Scheer and Burrow 2006), isomer B presents a higher electron affinity than isomer A. Looking at the LUMO energies, it’s more positive for isomer B which means that this isomer will be more accessible for capturing an incoming electron. Regarding the BDE of the C-Cl bond, it is weaker for isomer A, which therefore means that this bond would be more easily broken. These first results go in the direction of a more efficient ECNI ionisation for isomer B, except the calculation of the BDE. Regarding the ΔH°([M + Cl]) observed in LC-ESI-MS with Cl− enhanced conditions, isomer B forms more stable complexes than isomer A, which is consistent with the experimental RF observed in ESI.

5. Conclusions:
The quantum mechanical calculations generally agree with the observed instrumental results. Extending the range of positional isomers will be necessary in order to confirm the preliminary results obtained. At the end of this work, the observed results show that the GC-ECNI-HRMS coupling seems to be more interesting for CP analysis, with a larger observed homologue groups profile for the naturally contaminated samples. However, the LC-ESI-HRMS coupling still remain interesting for instrumental responses, which are less dependent on the chlorine content of the mixtures and less observed matrix effects.

6. Acknowledgments:
The research was funded by the French Ministry of Agriculture and Food Sovereignty.
**TUE-PM1-D1**  Towards a broad understanding of chlorinated paraffins response factors via the study of the LC-ESI-HRMS and GC-ECNI-HRMS couplings

7. References:

Introduction: Short-chain chlorinated paraffins (SCCPs, C_{10-13}) are considered to bioaccumulate, but data available on their biotransformation are scarce. Knowledge on medium- (MCCPs, C_{14-17}) and long-chain (LCCPs, C_{18+}) CPs is even more scarce (van Mourik et al., 2016). The existing studies have argued that CPs mainly accumulate in fatty organs whereas liver plays a role in their metabolism in rodents (Darnerud et al., 1982). Our previous work identified alcoholic, carbonyl and carboxylic biotransformation products of CPs in rat hepatic sub-cellular fractions. Unfortunately, their exact structures have not yet been identified because of a lack of reference standards. These Phase I metabolites are of great interest if they will be bioactivated and consequently, causing carcinogenicity. This study aims to synthesize reference standards of potential Phase I metabolites of CPs, which are important for both analytical and toxicological purposes.

Materials and Methods: The monohydroxy-CPs were prepared in the following steps: (1) preparation of Grignard reagents from alkyl or alkenyl alcohols, (2) subsequent reaction with alkenals to give unsaturated secondary alcohols (Grignard reaction), and (3) chlorination of the double bonds with chlorine gas produced in situ. Impurities were removed by silica gel chromatography, dry column vacuum chromatography (DCVC), recrystallisation, or combinations. The purified CP-alcohols were further oxidized by Dess-Martin oxidation to obtain the desired CP-ketones. The synthesized products were analyzed on GC/MS, and ^1H, ^13C and COSY NMR.

Results: We synthesized a range of monohydroxy-CPs and monocarbonyl-CPs (ketones) with a minimum purity of 98% (examples given in Figure 1). Purification was challenging especially for products of longer chain lengths and higher chlorination degree. DCVC was efficient and timesaving to remove the impurities. Concerning products of long chain lengths and high chlorine contents, recrystallisation could further increase the purity by ca. 2%. The synthesized products were structurally characterized by ^1H, ^13C and COSY NMR, and the purity was measured by GC/MS.

Discussion and Conclusion: With these synthetic routes, we have successfully synthesized a series of CP-alcohols and CP-ketones with designated chain lengths, chlorination patterns and functional group positions. Recrystallisation and flash silica gel chromatography are proven promising techniques for purification of these potential CP metabolites to a criterion of 98% purity, which enables the preparation of reference standards. The reference standards will contribute to analytical and toxicological studies on CP metabolism. In the near future, we will develop new protocols to synthesize other potential CP metabolites, such as dihydroxy-CPs, dicarbonyl-CPs, CP-aldehydes and CP-carboxylic acids.

Acknowledgments: We acknowledge funding from the European Union’s Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement REVAMP project No. 956374.

References:
Introduction:
Chlorinated paraffins (CPs) are multipurpose chemical additives with varying homolog composition used worldwide for almost a century. However, the homolog groups of short-chain CPs (SCCPs: C_{10–13}) with >48% chlorine (w/w) were globally restricted under the Stockholm Convention on persistent organic pollutants (POPs) in 2017, and products containing >1% SCCPs (w/w) should be considered as POPs [1]. Previous studies highlighted high mass fractions of SCCPs in technical CP mixtures from China not labelled as SCCPs [2]. Moreover, it has been reported that unintentional POPs may be formed during the production of CPs [3]. Thus, we aimed to determine the mass fraction of SCCPs, medium-chain CPs (MCCPs; C_{14–17}), and long-chain CPs (LCCPs: C_{>18}) in technical CP mixtures used worldwide and to assess the occurrence of dioxin-like impurities in them.

Materials and Methods:
36 technical CP mixtures used in various regions, including Europe, North America, South America, East Asia, South Asia, and the Middle East, were obtained from producers, importers or purchased as analytical standards. Manufacturing years varied from before 1975 to 2022. 74 CP homologs (C_{10–20Cl_{4–10}}) were quantified by LC–ESI–MS/MS. Unresolved CP contents were further assessed by LC–ESI–QTOF–MS. A DR-CALUX assay was performed to check dioxin-like activities in technical CP mixtures, and 10 samples with dioxin-like activities were selected for analysis of polychlorinated dibenzo-p-dioxins and furans (PCDD/Fs), and dioxin-like polychlorinated biphenyls by GC–HRMS.

Results:
In 20 samples, the CP homologs quantified by LC–ESI–MS/MS amounted to >81% (w/w); whereas in five it amounted to 55–77% (w/w); and in 11 to <50% (w/w) — being not detected in four of those. By further screening 375 CP homologs (C_{6–30Cl_{4–30}}), we identified very short-chain CPs (vSCCPs: C_{<10}), LCCP homologs >C_{20}, and highly chlorinated (Cl_{>10}) CP homologs in all samples with low or no resolved contents. At the end, one sample was mainly composed of vSCCPs, 10 of SCCPs, 13 of MCCPs, and 12 of LCCPs. C_{14Cl_{6}} and C_{11Cl_{6}} were the main homolog groups. 10 samples showed dioxin activity in the performed bioassay. However, only one sample had detectable levels of dioxins.

Discussion and Conclusion:
SCCP contents were measured from 80 to 100% (w/w) in seven technical CP mixtures. Notably, none of the commercial mixtures assessed were labeled as SCCP. On the other hand, the mass fraction ranges of MCCPs and LCCPs in this study were in the same range of those found in previous studies [3,4]. The dominance of C_{14Cl_{6}} and C_{11Cl_{6}} homologs were consistent with previous studies on technical CP mixtures, environmental matrices, and manufactured products [4,5]. The chlorine contents of SCCP homologs in all samples were calculated to be over 48% (w/w), which would classify almost 40% of our samples (n=14) as POPs under the restrictions of the Stockholm Convention. Some of these samples (n=3), which were recently produced and internationally traded, indicate a failure of certain manufacturing and importing country-Parties to comply with the Stockholm Convention restrictions [1]. However, other countries-Parties were found to have technical CP mixtures containing SCCPs below 1% (w/w), indicating the feasibility of producing CPs with lower SCCP contents if the manufacturers control the chain-length of n-alkane feedstocks used in CP production. PCDD/F levels in technical CP mixtures seem low, but other dioxin-like compounds need to be assessed to explain the dioxin-like activities detected in our technical CP mixtures. Indeed, Takasuga et al (2013) found polychlorinated naphthalenes to be the dominant unintentional POPs in products treated with CPs and in technical CP mixtures [3].

Acknowledgments:
This study was supported by the Environment Research and Technology Development Fund of the Environmental Restoration and Conservation Agency of Japan [grant numbers JPMEERF20193001 and JPMEERF20233001]. We thank the technical assistance of Mr. Humiaki Kato and Ms. Chieko Michinaka.

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AFTERNOON BREAKOUT SESSIONS I  TUESDAY 12 SEPTEMBER 2023

13:30 - 14:50

Fate, Detection and Analysis of Chlorinated Paraffins
M. Ricci & A. Fernandes

TUE-PM1-D4  Preparative Gas- and Liquid Chromatography for purification of synthesized Long Chain Chlorinated Paraffin standards

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Introduction:
Chlorinated paraffins (CPs) are a class of polychlorinated industrial chemicals and the technical products are grouped in three classes: short-chain (SCCPs, C10-13), medium-chain (C14-17), and long-chain CPs (LCCPs, C>17). Short Chain CPs (SCCPs) were classified as persistent organic pollutants (POPs) by the Stockholm Convention in 2007, followed by restrictions and bans. But production and use of Medium Chain CPs (MCCPs) and Long Chain CPs (LCCPs) is currently not regulated. Technical CP products consists of a complex mixture of thousands of different congeners. The validation of analytical methods for the identification and quantification of CPs in various matrices is being hindered due to a lack of certified reference materials (CRMs). One out of several goals of the REVAMP EU project is to develop CRMs for LCCPs with defined composition, which can be used to mimic the industrial mixtures and to be used for the certification of individual LCCPs in environmental and food samples and used in persistency and toxicity studies. The work we report here is the chemical synthesis of both individual LCCP congeners and LCCP single chain mixtures, and the purification/isolation of individual LCCPs or groups of LCCP isomers from the reaction mixtures by applying analytical approaches such as Semi-Preparative Liquid Chromatography (Semi-Prep-LC) and Preparative GC (Prep-GC).

Materials and Methods:
Fatty alcohols were first reacted with different Wittig salts to build up the alkenes which then were chlorinated using chlorine gas to make individual LCCPs congeners with defined chlorine position and chlorine content. LCCP single chain mixtures were prepared by chlorination of various lengths of n-alkanes with Sulfuryl Chloride under UV-radiation. Purification using flash column and recrystallization was used when that was applicable. The chemical purity was determined by GC-FID/MS, HPLC and NMR. NMR was also used for structure characterization. Further purification was performed with Semi-Prep-LC using H2O/ACN mobile phase on a X-bridge C18 5µm Column and Prep-GC using a DB-5MS Column on a Agilent 7820A GC system.

Results:
A series of LCCPs congeners and single chain mixtures has been synthesized ranging from C21-C25. Analytical purification methods such as Semi-Prep-LC and Prep-GC have been used to enhance the product purity and isolate pure LCCP congeners; as well as to separate LCCP-single chain mixtures into more precise groups of isomers, which are more suitable as reference standards for persistency, bioaccumulation and/or toxicity studies.

Discussion and Conclusion:
The synthesis approach of chlorination of n-alkenes gave LCCPs congeners with defined chlorine positions and chlorine content. However, other isomers and homologues can also be produced due to under- and/or-over chlorination with Cl2 gas. LCCPs isomers and homologues were difficult to remove by classical organic purification techniques. We developed Prep-LC Prep-GC methods for the purification / isolation of pure LCCP congeners This work builds on top of the earlier proof of concept paper separating MCCP mixtures resulting in standards suitable for further use in environmental sciences(Van Mourik et al., 2021).

Acknowledgments:
We thank Eureka-Eurostars programme, the EU Framework Programmes H2020-MSCA-ITN-2020 under the Marie Skłodowska-Curie grant agreement REVAMP project No. 956374, and H2020-INNOSUP, and the Norwegian research council for the fundings.

References:

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Introduction: Bioaccumulative perfluoroalkyl acids (PF AA) and precursors are found ubiquitously in the environment and are a large sub-group of synthetic compounds collectively known as per-/polyfluoroalkyl substances (PFAS). PFASs have been detected in several wild bird species, however, to date, there has been limited study of the potential physiological changes that PF AA may elicit in wild birds. Peregrine falcons (Falco peregrinus), apex predators of the terrestrial food web that predominantly consume birds, are known to accumulate persistent organic pollutants including pesticides, flame retardants and PFASs (Fernie et al. 2017, Sun et al. 2020). These chemical pollutants have been associated with thyroid-related changes in nestling peregrines across the Canadian Great Lakes Basin (e.g., Sun et al. 2021). In this study, our objectives were to determine the exposure of nestling peregrines to a broader suite of PFAS than previously measured, and by using metabolomics, conduct a novel and in-depth assessment of potential changes in the signature of endogenous metabolites of their thyroid system, steroid hormones, oxidative status, and other physiological measures.

Materials and Methods: Briefly, 35 peregrine nestlings were sampled at 22 ± 0.6 days of age in 2022, across the Greater Toronto and Hamilton Area (GTHA) of southern Ontario. Blood plasma was used to assess concentrations of 13 perfluorocarboxylic acids (PFCAs), 12 perfluorosulfonic acids (PFSAs), and 7 additional PFASs. Blood plasma was also used to assess up to 5 thyroid hormones (circulating, metabolites), 16 steroid metabolites, 11 thiol metabolites (oxidative stress), and 25 carnitines (fatty acids). The target PFC and PFS compounds were determined by ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) in negative electrospray mode (ESI-) (Letcher Laboratory). Statistical significance was p ≤ 0.05.

Results and Discussion: Detection rates of most individual PFSAs and PFCAs exceeded 94%, except for PF3OudS (57%), and were relatively low detection rates for 11Cl-PF3OudS (51%), N-EtFOSA (40%), N-MeFOSA (14%), HFPO-DA (11%), and FOSAA (11%). Similar to the PFAS profiles of other birds at lower trophic positions (e.g., tree swallows, Tachycneta bicolor) (Hopkins et al. 2023), the PFAS profile of the present nestling peregrines was dominated by PFOS > PFEtCHxS > PFNA. The peregrine chicks had higher plasma concentrations (mean ± SEM) of sum (Σ) 5PFSAs (19.4 ± 2.6 ng/g wet weight (ww)) than Σ13PFCAs (10.7 ± 0.53 ng/g ww), with PFOS concentrations (13.7 ± 2.3 ng/g ww) exceeding PFEtChxs (5.1 ± 0.1 ng/g ww), PFNA (2.1 ± 0.2), and the remaining 3 PFSAs and 12 PFCAs (> 1.2 ng/g ww). The dominance of PFOS in the profile of the nestling peregrines is consistent with previous reports on nestling peregrines sampled in 2017 (Sun et al. 2020), although some PFAS profile exceptions occurred in the chicks between both studies that may reflect the smaller urban geographic range of the present study (smaller) than previously (Sun et al. 2020). There were largely no differences in measured PFAS or biomarker concentrations between male and female nestling peregrines. In contrast to Sun et al. (2020), circulating TT3 was significantly correlated with Σ5PFSAs and Σ13PFCAs, and 7 individual PFAS congeners including PF3OudS. The thyroid hormone metabolites (T2, rT3, T3, T4) were not statistically correlated with any of the measured PFASs. Novel findings suggest that the metabolites of DHEA, and especially testosterone, were associated with multiple PFASs: DHEA negatively with 3 individual PFSAs and the Σ5PFSAs, in contrast to testosterone that was positively related to 5 individual PFCAs and the Σ13PFCAs. Thiol metabolites (oxidative status), especially GSH, cysteinylglycine, homocysteine, SAH and SAM, were repeatedly associated with PFEtCHxs, PFHxS, PFOS, Σ5PFSAs and Σ13PFCAs. The biological significance of these findings will be investigated and discussed.

Acknowledgements: We thank the Chemicals Management Plan and ECCC for funding and logistical support.

References:
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Introduction: For the past 7 decades, PFAS have been used and produced in many different products and applications, which has led to a widespread contamination of these compounds into the environment [1]. At this moment, not much is known about the effects of these compounds on avian wildlife [2]. Therefore, this study investigated associations between PFAS concentrations in the plasma of great tits and their oxidative status near a fluorochemical manufacturing facility in Antwerp.

Materials and Methods: Plasma samples were collected from great tits captured during the winter of 2022 on two sampling sites; one site closest to a fluorochemical manufacturing facility (3M) in Zwijndrecht, Belgium, which consists of both the 3M site and the neighbouring nature reserve Blokkersdijk and a site further away, Vlietbos. Blood samples were centrifuged and plasma and blood cells were stored separately. Plasma samples were analysed for 29 PFAS using UPLC-ESI-MS/MS. Afterwards, one biomarker of oxidative damage and ten (non-)enzymatic antioxidant biomarkers were measured in the red blood cells.

Results: The PFAS concentrations measured were among the highest ever reported in wildlife [3], with a mean PFAS of 16062 ng/mL at Blokkersdijk/3M and 375 ng/mL at Vlietbos. The PFAS profile of the plasma of the great tits consisted mainly of PFOS, PFOA, PFDA and PFDoDA, were concentrations were higher for these compounds closest to the plant. No signs of oxidative damage were seen since no significant change in malondialdehyde levels were measured, but some changes in antioxidant metabolites and enzymes were seen. The ferric ion reducing power and peroxidase levels were higher near the plant site, while the glutaredoxin concentration was higher further away. At last, positive associations were found between PFDoDA and glutathiones-S-transferase (GST), between PFOS and GST, between PFDA and peroxidase and between PFOS and peroxidase. In addition, a negative association was found between plasma PFDA concentrations and the total polyphenol content.

Discussion and Conclusion: Despite the phase-out of some PFAS, concentrations remain very high near the close proximity of the site. In contrast to this, great tits did not appear to suffer any change in plasma peroxidation – quantified as change in malondialdehyde - caused by the current contamination present in their plasma. Therefore, no clear signs of oxidative damage were seen, but some antioxidant defence responses were significantly upregulated by PFAS exposure. GST is responsible for detoxifying endogenous compounds such as peroxidised lipids and enables the breakdown of xenobiotics [4]. Our results suggest a rise in GST due to PFOS and PFDoDA exposure, which indicates the oxidative status of the birds has changed. Peroxidases on the other hand are a large group of enzymes that break up peroxides [4]. Our results suggest a rise in peroxidases due to PFDA and PFOS exposure, which indicates that the body suffers from a rise in peroxides due this exposure and therefore produces more peroxidase to counter this. At last, the total polyphenol content gives an idea of the amount of polyphenols present in the red blood cells. These have redox properties that allow them to act as antioxidants, and a decrease is seen due to PFDA present in the plasma. Overall, our results indicate that the great tits have managed to defend themselves against the possible oxidative damage coming from PFAS contamination. In addition, since this was a short-term experiment, the potential cost or effects of these upregulated antioxidant defences could not be investigated. More experimental data should be gathered to investigate the specific pathway in which PFAS induce oxidative stress in avian species.

Acknowledgments: We thank Peter Scheys to help with the placing of the nest boxes and Tim Willems for the UPLC-ESI-MS/MS analysis. We also thank Natuurnpunts and 3M for allowing us to perform part of our study on their premises. This project was funded by the Research Foundation Flanders (FWO) in terms of a junior postdoctoral grant (12ZZQ21N) to TG. Ethical permission to capture and handle the birds was provided by the Ethical Committee of the University of Antwerp, as well as by the Flemish Animal Welfare Council (EC2020-58).

References:
Introduction: The bioaccumulation and tissue distribution of perfluoroalkyl carboxylates (PFCAs) are of great concerns due to their unique properties. Several proteins and phospholipid have been proposed as important factors affecting the tissue distribution process of PFCAs. However, the explicit roles played by specific chemical properties of PFCAs, the toxicokinetic of PFCAs during bioaccumulation process, and the interaction between PFCAs and biological tissues are not yet elucidated or not fully clarified. The present study was want to answer above concerns.

Materials and Methods: Twenty five laying hens were exposed to C4-C18 PFACs with different treatments (high and low dose sustain exposure groups, high and low dose exposure-depuration group, and control group). Eggs, serum sample were collected during the experiment process and finally various tissues and organs were collected at the end of the experiment. PFCA were determined using ultra-performance liquid chromatography (UPLC-MS/MS).

Results: The gastrointestinal absorption efficiencies (30 to 44%) of short-chain PFCAs (C4–C8) were remarkably lower than those (76 to 90%) of long chain PFCAs (C9–C18).The uptake kinetic of PFCAs in the hens can be divided into three groups according to carbon chain length. The first group (C4–C8) first increasing then decreased, the third group (C18) show increased trend until end of experiment, and the second group (C9–C16) show a fluctuating decreasing trend. The depuration rate exhibited an inverted U-shape relationships with carbon chain. C18 show the greatest bioaccumulation potential. The concentration of PFCAs positively related to phospholipid content of tissue/organs. The order of PFCAs among tissue in uptake phase was different from that of depuration phase. The egg-maternal ratio (EMR) calculated by matched egg and serum sample indicated that EMR increased with increasing carbon chain length from C7–C11, and reached a plateau phase for C12–C18.

Discussion and Conclusion: This studies further verified the key role played by phospholipids on the tissue distribution and maternal transfer of long-chain PFCAs. The influence of carbon chain length on distribution of PFCAs in different tissues of hens was demonstrated. A tissue-specific affinity to specific carbon-chain PFCAs was revealed. The highest PFCAs levels found in eggs (yolk) cautions the potential PFCAs exposure risk by egg consumption. The high conservation of C14 in brain and C18 in main tissues of hen highlights the importance of further surveying on ecotoxicology and human health risk of these much longer carbon chain PFCAs. The present study helps elucidate the interaction between PFCAs and biological tissues, and provides toxicokinetics in bioaccumulation process of PFCAs.

Acknowledgments: The study was funded by the National Nature Science Foundation of China (Nos. 41877386, 41931290, 42277267), and Local Innovative and Research Teams Project of Guangdong Pearl River Talents Program (2017BT012134) and Guangdong Foundation for the Program of Science and Technology Research (Nos. 2020B1212060053 and 2019B121205006).
Per- and Polyfluorinated Substances (PFAS): Toxicity
P. Leonards & JH. Yang

TUE-PM2-A4  In vitro toxicity profiling and relative equivalent potencies (RPF) of a large number of PFAS on a tailored panel of effect- and human-cell based bioassays

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BioDetection Systems BV, Science Park 406, 1098 XH, Amsterdam, the Netherlands,

1. Introduction:
PFAS are widely distributed in the environment and are a potential risk for human and animal health. The European Commission’s zero pollution vision for 2050 aims at reducing air, water and soil pollution to levels no longer considered harmful to health and natural ecosystems, that respect the boundaries with which our planet can cope thereby creating a toxic-free environment. The in vitro toxicity potency of 41 PFAS compounds and one technical product (ADONA) was evaluated on a tailored-set of 6 CALUX bioassays for cytotoxicity (Cytotox CALUX), oxidative stress (Nrf2 CALUX), obesity/fat metabolism (PPARγ/anti-PPARγ CALUX) and thyroid hormone disruption (anti-TRβ and TTR-TRβ CALUX). Here we used in vitro toxicity profiling for ranking toxicity and to establish relative potency factors (RPFs) that e.g., can be used to convert chemical derived concentrations into total PFOA equivalents and subsequently for direct comparison to e.g., biologically derived TTR-TRβ CALUX results. In the present study, the TTR-TRβ CALUX (disruption of TTR-T4 binding) was found to be the most responsive bioassay for most PFAS compounds studied. The results indicate that a combination of effect-based analysis using e.g., the TTR-TRβ CALUX reporter gene bioassay and targeted congener specific chemical analysis would be a suitable strategy to improve the assessment of the whole complex mixture of known and yet unknown PFAS and their transformation products in the environment, wildlife and for public health.

2. Materials and Methods:
Materials: 41 PFAS compounds and one technical product (ADONA) were obtained from Campro Scientific and Da Vinci (see Table 1).

Methods: Most of the CALUX bioassays were performed as described earlier (Sonneveld et al. 2007). The TTR-TRβ or PFAS CALUX bioassay was carried out under conditions described in detail previously (Collet et al., 2020; Behnisch et al. 2021; Behnisch et al., 2022). Following a wide-panel CALUX screening (n=12) of the most common PFAS, the most responsive bioassays (n=6) have been selected for PFAS toxicity profiling and activity ranking. Routinely executed CALUX bioassays have been automated using a compact liquid handling system (Hamilton StarLab) and luminometers with stackers (Berthold Mithras and Tecan Infinite). Serial dilution series of PFAS have been analyzed in duplicate and each analysis consisted of triplicate well-plate testing. In vitro toxicity was quantified using the lowest concentration showing activity in the concentration-response curves (PC5 for agonistic and PC80 for antagonistic activity; IF-1.5 for non-receptor mediated activity such as Nrf2 CALUX).

3. Results and Discussion:
Several toxic effects of PFAS on humans are mediated through nuclear hormone receptor interactions (NHRs). So far, a limited number of PFAS are reported to have endocrine disruptive potential, interfering with both the thyroid and steroid hormone systems (see for overview at Behnisch et al. 2021). Effects on steroid hormones include changes in estrogenic, androgenic and thyroidogenic activity (Sonneveld et al. 2007; Young et al. 2021). Additionally, many PFAS interfere with the activity of peroxisome proliferator-activated receptor alpha (PPARα), a mechanism associated with tumour development in the liver, pancreas and testicles. PFAS also activate the PPARγ, a receptor which plays a role in preadipocyte differentiation into adipocytes. Thus, PFAS may be involved in the development of obesity through affecting this receptor. For general toxicity purposes, also cytotoxicity (Cytotox CALUX) and oxidative stress (Nrf2 CALUX) activities in the cell have been tested for the selected 41 PFAS compounds and one technical product (ADONA).

In the first step, twelve out of the 41 PFAS compounds and one technical product (ADONA) have been screened on a wide panel of 12 CALUX bioassays (such as for PPAR, endocrine (EAT) testing, genotoxicity p53 related and general toxicity pathways, e.g., early warning PXR) to characterize them for their in vitro toxicity. The most promising bioassays e.g., the PFAS CALUX (TTR-TR), anti-TRβ, PPARα, anti-PPARγ, Nrf2 and Cytotox CALUX bioassay were then selected for the remaining 30 PFAS compounds/technical mixture in duplicate.

For the 41 PFAS compounds and one technical product (ADONA) tested on the selected CALUX battery, the TTR-TRβ or PFAS CALUX showed to be the bioassay on which most compounds elicited activities (33 active compounds). Next, the PPARα CALUX bioassay showed most activity (21 active compounds) followed by the anti-TRβ (17 active compounds), oxidative Nrf2 (11 compounds), anti-PPARγ (10 active compounds), and cytotox CALUX bioassay (10 compounds). 13 PFAS compounds showed activity in only 3 CALUX bioassays.
In vitro toxicity profiling and relative equivalent potencies (RPF) of a large number of PFAS on a tailored panel of effect- and human-cell based bioassays

Toxicity profiling of these 42 PFAS standards using a tailored set of *in vitro* CALUX bioassays showed that the PFAS CALUX bioassay is the most responsive and sensitive bioassay as compared to the other mode of actions evaluated. In figure 1, typical dose-response curves using the PFAS reporter gene for several PFAS are presented.

The *in vitro* established relative potency factors (RPF) of the tested PFAS relative to PFOA (RPF = 1) in the PFAS CALUX varied between 0.00015 (6:2 FTAB) and 2.3 (P37DMOA). In table 1, we present the various *in vitro* derived RPF-values obtained from the here used CALUX panel as well as the currently in Europe discussed *in vivo/in silico* read-across derived RPFs (EU-COM 540, 2022; see also Bil et al. 2021). The PFAS reporter gene assays show relative potencies for most PFAS in the same order of magnitude as the *in vivo* RPFs, but differs strongly for some other, such as for PFNA to PFDoDA, which may be due to e.g., differences in kinetics. Also, very active thyroid hormone disrupting chemicals such as P37DMOA (RPF = 2.3) and PFOSA (RPF=0.75) are yet lacking from the EU-COM proposal.

4 Conclusions:
We were able to demonstrate here that sensitive effect- and human cell-based *in vitro* toxicity based bioanalysis tools can play an important role to assess the *in vitro* toxicity of the whole group of the few known and many unknown PFAS-chemicals and mixtures thereof. This data shows that the most promising CALUX bioassay is the PFAS CALUX, which is based on their common property to bind to specific thyroid hormone transport proteins and thereby interfering with the thyroid-hormone system with possible adverse health consequences. *In vitro* relative potency factors determined following PFAS CALUX bioanalysis of a large set PFAS indicated more relevant yet unknown PFAS compounds. Our studies indicate that not only the now proposed 24 PFAS (EU-COM 540, 2022) show *in vitro* toxic properties, but also additional PFAS (PFOSA and P37DMOA) need to be strongly considered. This data indicates that *in vitro* toxicity analysis of total PFAS content in environment, food or public health related matrices using in vitro bioassays is a promising and suitable strategy to cover complex mixtures of the handful yet known versus the thousands of yet unknown PFAS.
**Per- and Polyfluorinated Substances (PFAS): Toxicity**

*P. Leondards & JH. Yang*

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**Table 1:** Listing of *in vitro* derived RPF-values of this study and of in vivo/in silico read-across derived RPF-values (EU-COM 540, 2022; see also Bil et al. 2021). --- = no activity; na = not analysed.

<table>
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<tr>
<th>Bioassay</th>
<th>cytotox CALUX</th>
<th>Nrf2 CALUX</th>
<th>PPARα CALUX</th>
<th>anti-PPARα CALUX</th>
<th>anti-TRβ CALUX</th>
<th>TTR-TRβ CALUX</th>
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<td>RPF (-)</td>
<td>RPF (-)</td>
<td>RPF (-)</td>
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TUE-PM2-A4 In vitro toxicity profiling and relative equivalent potencies (RPF) of a large number of PFAS on a tailored panel of effect- and human-cell based bioassays
TUE-PM2-A4  In vitro toxicity profiling and relative equivalent potencies (RPF) of a large number of PFAS on a tailored panel of effect- and human-cell based bioassays

6. Acknowledgments:
The in vitro toxicity testing and resulting RPF values are a product of the PROMISCES Project, which runs from November 2021 to May 2025 and is funded by European Union’s Horizon 2020 research and innovation program under Grant Agreement No 101036449.

7. References:
TUE-PM2-B1  Biomonitoring of bisphenols and organophosphate flame retardants in Japanese children and association with immune response outcomes: The Hokkaido Study

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Introduction: As chemicals in consumer products continue to be regulated, alternative compounds are increasingly introduced into our environment. Analogous bisphenols (BPs) such as bisphenol F (BPF) and S (BPS) are currently replacing bisphenol A (BPA), while various organophosphate esters (OPs) are applied in favour of older, more persistent flame retardants and plasticizers. Recently, the European Food Safety Authority (EFSA) drastically lowered the tolerable daily intake (TDI) for BPA to 0.2 ng/kg body weight per day and concluded that there is a health concern from dietary BPA exposure in all age groups (1). Several animal and epidemiological studies have reported significant associations between BPA or OPs exposure and health effects, e.g. allergy symptoms and altered hormone levels. While exposure to these contaminants of emerging concern is increasing in humans, studies on their alteration of the immune response are scarce. Biomonitoring is a valuable tool to investigate total internal exposure and evaluate potential health risks, which might be more pronounced in children. Therefore, this study aimed to assess the exposure of Japanese children to BPs and OPs and examine the association with infectious diseases and antibody levels after vaccination.

Materials and Methods: This study was part of a prospective birth cohort (Hokkaido Study). Spot urine samples from 9- to 12-year old children (n = 427) were collected from 2017 to 2020 and analysed for seven BPs and thirteen OP metabolites. Diphtheria and tetanus antibody concentrations were measured in serum. Demographic characteristics and history of infectious diseases of the study population were acquired through a face to face survey. Associations between contaminant concentrations and health outcomes were evaluated using multiple linear regression, adjusted for age, sex and days since last vaccination. Estimated daily intakes (EDI) for each contaminant were calculated and compared to available TDI or reference dose (RID) values.

Results: Nine measured chemicals were detected in more than 50% of urine samples, namely: BPA, BPF and BPS, diphenyl phosphate (DHP), bis(1,3-dichloro-2-propyl) phosphate (BDCIPP), bis(2-butoxyethyl) phosphate (BBOEP), 1-hydroxy-2-propyl bis(1-chloro-2-propyl) phosphate (BCIPHP), 2-ethylhexyl phenyl phosphate (EHPHP) and 2-hydroxyethyl bis(2-butoxyethyl) phosphate (BOEHEP). Children presenting higher BPF concentrations in urine showed significantly lower tetanus antibody levels (β, 95% CI = -1.07; -1.12, -1.02), while children with a higher molar sum of tris(2-chloroisopropyl) phosphate (TCIPP) metabolites in urine showed significantly lower diphtheria antibody levels (β, 95% CI = -1.18; -1.30, -1.06). Higher urinary BPA concentrations were significantly associated with higher odds (OR, 95% CI = 1.48; 1.17, 1.89) of developing high fever during the last year in Japanese children. Similarly, BPF showed higher odds of high fever, however statistical significance was borderline (OR, 95% CI = 1.12; 0.98, 1.29). All children with a measurable BPA level (70%) showed an EDI (range 5.5 – 1099.5 ng/kg body weight/day) higher than the recent TDI.

Discussion and Conclusion: The main BPs (BPA, BPF and BPS) showed median and maximum concentrations in the same range as our previous study in 7-year old children from the same birth cohort (2). Detection frequency (DF) of BPA was slightly lower in the current population, which could be explained by further phase out of BPA in recent years and/or different age-related behaviour. DF and median concentrations of OP metabolites were slightly lower compared to the measurements in 7-year old children (3). Higher BPA and BPF levels showed higher odds of developing a high fever, and higher BPF levels also associated with lower tetanus antibody levels, which may suggest immunosuppressive effects. Since BPA is still responsible for the highest median urinary concentration of all measured BPs, its effects are likely more pronounced. This study indicates that school children in Japan are extensively exposed to multiple consumer product chemicals, which could potentially result in health effects. The findings warrant further research on these classes of contaminants of emerging concern.

References:
Introduction: Diet is considered the primary source of exposure for endocrine-disrupting chemicals (EDCs) to the general population, which include plasticizers, synthetic preservatives, industrial chemicals and environmental pollutants (Diamanti-Kandarakis et al., 2009). EDCs are synthetic chemicals that are used in everyday products that can interfere with the body’s natural hormones and produce a range of diseases in humans. Despite their extensive applications, EDCs are characterized as highly concerning compounds due to their capacity to migrate into food items from food packaging materials. Based on new evidence, The European Food Safety Authority (EFSA) recently re-evaluated the tolerable daily intake (TDI) for bisphenol A (BPA) from 4000 ng/kg bw/day to 0.2 ng/kg bw/day and the United States Environmental Protection Authority (US EPA) has proposed a maximum contaminant level of 4 ng/L for perfluorooctanoic acid (PFOA) in drinking water (USEPA, 2023, EFSA, 2023). The intake of nonalcoholic beverages accounts for a large and increasing volume of personal consumption among children and adults worldwide. Hence, the assessment of nonalcoholic beverages as sources of human exposure to EDCs is a key and foremost important issue for consumer safety.

Materials and Methods: PFAS (n = 63), bisphenols (n = 14), parabens (n = 13), benzophenone-type UV-filters (n = 8), biocides (n = 3), alkylphenols (n = 2), and nitrophenols (n = 2) plus thirty-six mass-labelled surrogates were assessed. In total, 162 products spanning 87 brands from 16 countries were sampled and grouped into four categories: soft drink (n = 56), packaged water (n = 53), coconut water (n = 16), sports drinks (n = 10), energy drinks (n = 22). The 105 analytes were analysed at ng/L levels using optimised solid-phase extraction or liquid-liquid extraction methods and targeted liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Results: Among the 162 beverages investigated, 144 products (90%) contained at least one targeted compound. The total concentration of EDCs in products ranged from n.d. to 90,288 ng/L, with the highest concentrations (mean: 2677 ng/L, 95th percentile: 11,838 ng/L) found in aluminum and tinned coconut waters. Of the 105 EDCs analysed, 65 compounds were found with at least one positive detection in samples. BPA reported the highest concentration of all targeted analytes in coconut waters with consistently high levels ranging from 0.5–27,708 ng/L. Interestingly, two structural isomers of BPA, tentatively termed BPA1 and BPA 2, were identified in 14% and 12% of samples, respectively, at higher concentrations than BPA (up to 41,000 ng/L). Artesian water also reported the highest level of PFOA at 14 ng/L. Aluminum and tinned products were found to contain significantly higher concentrations of EDCs compared to that of plastic (PET), recycled plastic (rPET), glass and carton (TetraPAK) products (p < 0.001). The estimated daily intake (EDI) of mean BPA concentrations from beverages for adult males and females (≥18 years old) and children (2-17 years old) were calculated and ranged between 7.94 to 21.34 ng/kg/bw/day.

Discussion and Conclusion: Our results show that aluminum and tinned packaged beverages contain significantly higher levels of EDCs than glass, carton, or plastic products. Calculated EDIs suggested that exposure to BPA through beverage consumption was much higher (up to 100-fold) than the recently evaluated TDI. Additionally, PFOA exceeded the newly drafted US EPA drinking water guideline in packaged water. Our results suggest that daily consumption of certain beverages may present a human health risk to children and adults. As BPA isomers are beginning to be reported in the literature, future studies are warranted to corroborate these findings.

References:
Environmental and Human Exposure to Plasticizers and Other Consumer Product Chemicals

G. Poma & C. Pirard

TUE-PM2-B3  Non-targeted Screening of Chemicals Migration from Plastic Food Contact Materials by High Resolution Mass Spectrometry

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(1) Mass Spectrometry Laboratory/Organic Pollutants, (2) Environmental and Water Chemistry for Human Health (ONHEALTH), Institute of Environmental Assessment and Water Research, IDAEA-CSIC, Jordi Girona 18, 08034 Barcelona, Spain

1. Introduction:
Food packaging has become essential to preserve the nutritional properties and sensory characteristics of food. Despite the advantages that packaging provides to the consumer, nowadays there is concern in environmental and health topics since food packaging could represent a potential source of contamination because of the possible transfer of components from packaging to food.

The aim of this study is to investigate migration of chemicals from plastic food contact materials (FCMs) used for microwave and conventional oven heating (roasting bags, microwavable plastic trays and microwave oven bags). The study focuses on foods that are marketed in packaging to be cooked with (vegetables, purée, omelette and meat), in order to evaluate if any chemical migrates from FCMs only by contact or when the food has been cooked.

2. Materials and Methods:
Methanol (MeOH), dichloromethane (DCM), n-hexane (Hx), ethyl acetate (AcOEt) and isoctane Suprasolv; acetonitrile (ACN) and water (H2O) LiChrosolv, hydrochloric acid (HCl) (25%), formic acid (HCOOH) (98-100%) EMSURE, were supplied by Merck (Darmstadt, Germany). Ammonium hydroxide (NH4OH) (25%) was purchased from Fluka. Sodium hydroxide pellets (97%) was provided by Carlo Erba Reagents GMBH (Emmendingen, Germany). Sodium sulphate anhydrous (12-60 mesh) was supplied from J.T. Baker. Silica gel 60 (0.063-0.200mm) and florisor (0.150-0.250mm) were acquired from Sigma-Aldrich.

Two types of samples were analyzed in this study: food -broccoli, asparagus, potato, chicken, pork loin, omelette and eggs- and its plastic containers -LDPE: oven and microwave bags; and HDPE: microwave trays.

Due to the variety of samples studied and the respectively post instrumental analysis, several extraction processes have been carried out [1, 2, 3]. Each plastic container was extracted by ultrasonic-assisted extraction (USAE) using three different solvents: methanol, ethyl acetate and a mixture of dichloromethane: hexane. These solvents were selected in order to cover a wide range of compounds depending their polarity.

In the case of food, analysis was carried out from the food in bulk, the food in contact with the plastic container and the food after cooking it. All of them subjected to real consumption cooking conditions. For conventional oven treatment samples were placed inside the container for 30 min at 180°C and for microwave treatment for 5-10 min at 800W.

The extraction of food was performed by USAE and soxhlet extraction.

For the analysis of semivolatile and nonpolar compounds, USAE was used to the treatment of broccoli and potatoes with AcOEt; asparagus purée with DCM and omelette and egg with a mixture of Hx:DCM. Meat samples (chicken and pork loin) were extracted with soxhlet procedure with a mixture of Hx:DCM. The extracts were clean-up with sodium sulphate anhydrous, florisor or neutral silica. Final volume and solvent were adjusted for gas chromatography (GC) analysis.

Additionally, for the analysis of polar compounds, an USAE with MeOH -ACN for eggs- following by SPE clean-up step with Oasis HLB cartridges (6cc, 200 mg) was performed for all foods for later analysis by liquid chromatography (LC)-high resolution mass spectrometry (HRMS).

LC and GC separation systems coupled with Q-Orbitrap mass analyzers were used for comprehensive non-targeted screening. This approach expands the chemical space covered to include semivolatile, nonvolatile, nonpolar and polar compounds.

For LC-HRMS analysis, a Q-Exactive hybrid Quadrupole-Orbitrap mass analyzer (Thermo Fischer Scientific, Bremen, Germany) equipped with a heated electrospray ionization (H-ESI) source was used. The chromatographic separation was achieved using an Acquity LC system (Waters, Milford, MA, USA) equipped with a C18 analytical column, Kinetex XB C18 (100mm×2.1mm, 2.6 µm) purchased from Phenomenex (Torrance, CA, USA). The mobile phase was composed of by HPLC-water (eluent A) and methanol (eluent B). The gradient profile was started at 95% of eluent A, and this composition was kept constant for 2.5 min: then ramped to 35% of this, held for 10 min, then it was decreased to a 0% A for 5 min was returned to 95% A to the initial conditions in 0.5 min, followed by a re-equilibration time of 10 min. For separation of the compounds, flow rate was set at 0.2 mL/min, and the injection volume was 10 µL. H-ESI source parameters used: 40 psi sheath gas, 10 a.u. (arbitrary units) auxiliary gas, and 300°C probe heater temperature, with an S-lens RF level at 60%, in separate inject. In positive mode 3 kV spray voltage and 400°C capillary temperature and in negative mode -2.5kV and 300°C were utilized. Nitrogen that was used as drying gas in electrospray source with 99.995% purity was obtained from Air Liquide (Spain).
All samples were run in positive and negatives electrospray ionization modes in full MS at 70,000 of Full Width Half Maximum (FWHM) resolution for \( m/z \) 100-1000 in both modes. Automatic gain control (AGC) was set at 1×10^6 counts with a maximum injection time (IT) of 250 ms. Based on the acquired full scan information, inclusion and exclusion lists were generated and used for the following instrumental run in data dependent (dd)MS2. For ddMS2, the resolution was 17,500 FWHM, AGC target 1×10^5 counts, maximum IT 50 ms, isolation window 2 m/z and normalized collision energy 30V.

The GC-HRMS analysis was performed on a Q-Exactive Orbitrap MS coupled with Trace 1310 GC and TriPlus RSH autosampler from Thermo Fisher Scientific (Bremen, Germany). GC oven temperature program started at 50 °C, held for 2 min, followed by a ramp at 20 °C/min to 150 °C, the second ramp at 6 °C to 320 °C, held for 10 min. The separation was performed on a DB-5 30 m x 0.25 mm x 0.25 μm column from Agilent J&W with carrier gas flow (helium) at 1.5 mL/min. MS was operated in a scan mode for \( m/z \) 50-750 at 60,000 FWHM in electron ionization (EI) at 70 eV. AGC target: 10^6 counts, emission current 50 μA and temperature of 280 °C for source and transfer line.

Compound Discoverer 3.3 software was used for data handling and identification. Workflow criteria were established in GC-HRMS and LC-HRMS: accurate mass data (<5ppm), isotope pattern matching, Total ion current (TIC) threshold (10^4), mark background compounds (ratio sample/blank 5), ion overlap (98%), etc [4, 5, 6, 7]. After data processing, filters were applied to reduce the huge number of compounds tentatively identified, such as group areas (10^4), HRF score (>80%), SI (>700), RSI (>700). A manual review of the assigned compounds was performed to refining the results. The compounds were tentatively identified by searching NIST Mass Spectral Library (GC-HRMS) and mzCloud and ChemSpider (LC-HRMS).

This approach allowed identifying migrated chemicals- intentionally added substances (IAS), used in the production of polymeric materials and plastics, and non-intentionally added substances (NIAS) such as degradation of IAS.

### 3. Results:
A large number of compounds were tentatively identified in all samples. Tables 1 and 2 show the most significant.

The compounds found in the uncooked food are compared with those found in the cooked food, as they are directly related. The last column of the tables shows the migration limits established in Regulation No. 10/2011 for the substances included in its Annex I [10]. Results for bulk food are not presented because they are not significant.

### Table 1: Identified migrated compounds by LC-ESI (+/-)-HRMS

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<th>Potato (MW)</th>
<th>Omelette (MW)</th>
<th>Chicken Oven</th>
<th>Loin Oven</th>
<th>SML (mg/Kg)</th>
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<td>Surfactant, emulsifier, lubricant</td>
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Table 1: Identified migrated compounds by LC-ESI (+/-)-HRMS

✓ identified in not cooked food;  ✓ identified in cooked food; MW: microwave.

SML: specific migration limit; *GML: generic migration limit 60mg/Kg. [10]
A total of 39 chemical compounds that migrated significantly from plastic packaging to food, either by direct contact or after cooking, were identified. Phthalates, acids and esters compounds were mainly migrated just with contact during storage. Instead, after cooking, the presence specifically of glycerol and erucamide was detected. The surfactant 2-[2-(dodecyloxy)ethoxy] ethyl hydrogen sulfate was only detected when cooking in an oven and the hexadecanoic acid methyl ester only when microwave cooking. In general, methyl ester compounds have been found in all foods that have been cooked in the microwave, regardless of the packaging used.

We can differentiate two groups of substances characteristic of LDPE and HDPE containers, respectively: erucic acid and hexanediol acid mono(2-ethylhexyl) ester were observed only in oven and microwave bags (LDPE containers); and 9,12-octadecadienoic acid (z, z) and octyl 3,3,3-trifluoropropanoate only in microwave trays (HDPE containers). In LDPE packaging (microwave and oven roasting bags) a greater number of observed plasticizers were migrated. All semivolatile compounds identified occur in both uncooked and cooked foods. Much less semi-volatile compounds have been identified in not cooked food; identified in cooked food; MW: microwave. SML: specific migration limit; *GML: generic migration limit 60mg/Kg. [10]

4. Discussion:

The type of cooking (microwave or oven), container (tray HDPE or bag LDPE) and food (vegetables, purée, omelette and meat) will influence the compounds that can migrate.

According to Commission Regulation (EU) 10/2011 [10], compounds such as phthalates, glycols, acids and butyl hydroxy toluene have a specific migration limit in mg of substance per kg of food or generic migration limit of 60 mg/Kg. In addition, “non-detectable migration” is considered for potentially migrating substances (IAS and NIAS), with a migration <10µg of substance per Kg (Article 13 and Annex 1). Taking these migration limits into account, we will work on the quantification of the degree of migration of the compounds that we have qualitatively detected, to determine if they are within the established limits. Our findings are in accordance with similar published studies. Abietic, myristic and other acids, erucamide, glycols and phtalates are chemicals frequently identified in plastic products [4-9]. Styrene determined in oven bags (LDPE) in the present work was also found in all polystyrene samples in previous studies and butylated hydroxytoluene from omellette microwavable tray in polypropylene, PVC and polyurethane [8]. On the other hand, 2,6-diterbutylphenol observed in all studied containers except LDPE bag of broccoli was also detected in bioplastic such as poly(lactic acid) (PLA) and poly(ethylene butyrate) (PBE) by other authors [7]. As well as, abietic acid, disobutylphthalate, erucamide and nonanoic acid tentatively identified in some LDPE containers. Hexanediol acid mono(2-ethylhexyl) ester was identified in PVC and HDPE samples and 9-octadecenoic acid (z)-methyl ester in LDPE and PVC ones [9].
Phthalates promote obesity in cell and animal models, particularly, diisobutyl and benzyl butyl phthalate observed in bag LDPE of potato are also tentatively identified as metabolism-disrupting chemicals [9]. Some compounds tentatively identified in this work have been described as inducers of antiandrogenicity, estrogenicity, oxidative stress response or cytotoxicity: butylated hydroxytoluene, benzylbutyl phthalate and 9-octadecanoic acid (z)-methyl ester [8]. Finally, it should be mentioned that some plastic compounds, such as tartaric acid and triethyl phosphate for HDPE containers or tereftalic acid for LDPE containers have not migrated into the food either by contact or by cooking under tested conditions. Therefore, these types of substances are safe for the consumer and containers with these components can be used minimizing the risk of migration.

6. Acknowledgments:
This study was supported by the Spanish Ministry of Science and Innovation (Project EXPOPLAS (PID2019-110576RB-I00), Grants CEX2018-000794-S and PRE2020-093018 funded by MCIN/AEI/10.13039/501100011033 and by “ESF Investing in your future”), and by the Generalitat de Catalunya (Consolidated Research Group 2021 SGR01150).

7. References:
TUE-PM2-C1 Re-evaluation of WHO toxic equivalency factors (TEFs) for PCDD/Fs and PCBs

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1. Introduction
The WHO has organized expert consultations over the past 2-3 decades with the objective to harmonise TEFs for dioxins and dioxin-like compounds at an international level and to provide recommendations to national regulatory authorities. The TEF values assigned to individual PCDD/Fs and PCBs express the toxicity of individual congeners relative to the most toxic form of dioxin, 2,3,7,8-TCDD. The previous WHO TEFs for dioxin and dioxin-like compounds were established by WHO through an expert consultation in 2005, with a stated intention that these should be revised periodically (van den Berg et al, 2006). To address this, WHO convened another ad-hoc expert consultation in Lisbon, Portugal from 17 to 21 October 2022 during which the 2005 WHO toxic equivalency factors (TEFs) for dioxin-like compounds, including some polychlorinated biphenyls (PCBs), were re-evaluated. The objective is to harmonize the TEFs for dioxins and dioxin-like compounds at an international level, thereby giving recommendations to national regulatory authorities. TEF expresses the toxicity of dioxins, dibenzofurans and dioxin-like PCBs relative to the most toxic form of dioxin, 2,3,7,8-TCDD.

2. Materials and Methods
Describe what you have done in this work: List all chemicals, equipment or materials (do not use proprietary names) used in this study and present in the same sequence as results will be shown in the next section. Where possible, refer to established methods or procedures. Do not include comments, results or discussion in this section.

Since the previous 2005 consultation, a large amount of congener-specific data has been published on estimates of relative potency (REPs) and potential TEF selections. These relative potency data from individual studies have been added to an updated REP database, and these data were used in a Bayesian meta-regression approach to compare the evaluations to existing TEFs and to decide whether changes would be warranted. The methods applied for this TEF re-evaluation directly follow recommendations made by the 2005 WHO expert consultation.

Preceding the 2022 expert consultation, WHO worked for more than two years with a group of international recognized dioxin experts. Based on the recommendations of this group, and with the support and collaboration of the European Food Safety Agency (EFSA) - two contractors, ToxStrategies and KeyToxicology, were engaged. A refined database of relative potency estimates was prepared, in order to develop a consensus-based REP weighting, and a peer review of the new data added to the REP database was conducted. To further validate the data and models used to refine the TEF values, WHO engaged with experts from the U.S. National Institute of Environmental Health Sciences (NIEHS) to conduct a comprehensive assessment of the Bayesian methods and its application to the REP database. This methodology and level of peer review is unprecedented in the evolution of the REP database, and provides additional confidence in the data that 2022 WHO experts used for making recommendations for changes to TEF values. The background data and models used to derive these updated 2022 TEF values will be published in the peer-reviewed literature after the WHO has published the 2022 updated WHO TEF values.

3. Results
There was consensus among all the invited experts that the updated REP database indicated a need to reevaluate the 2005 WHO TEF values for dioxins, furans and dioxin-like PCBs. The Bayesian method was used to validate the REP database which resulted in higher confidence and certainty in the outcome of the 2022 expert consultation.

It was concluded that the selection of dioxin-like compounds at the 2005 WHO meeting was still relevant and needed to be revisited for possible changes in TEF values. The use of new data in combination with the Bayesian approach resulted in an update of almost all 2022 WHO TEFs values when compared to the 2005 list of TEF values.

4. Conclusions
If the revised TEFs were to be used in a risk assessment concept, it can be concluded that this would include a moderate reduction of total dioxin-like toxic equivalencies (TEGs) for chlorinated dioxins, dibenzofurans and PCBs.

The outcome and details of this expert consultation will be published in a peer-reviewed paper in 2023.

5. Acknowledgments
This work reflects the outcome of work conducted by the organisations mentioned in the text and by the following individuals who participated in the meeting convened by WHO. Michael DeVito, Bas Bokkers, M.B.M. van Duursen, Karine van Ede, Mark Feeley, Elsa Antunes Fernandes Gaspár, Laurie Haws, Sean Kennedy, Richard Peterson, L Ron Hoogenboom, Keiko Nohara, Kim Peterson, Cynthia Rider, Martin Rose, Stephen Safe, Dieter Schrenk, Matthew W Wheeler, Daniele S. Wikoff, Bin Zhao and Martin van den Berg.

6. References
1. Introduction:
The European Food Safety Authority (EFSA) is an independent body providing scientific advice and communication on risks associated with the food chain according to Regulation (EC) No 178/2002. As a risk assessor, EFSA produces scientific opinions and advice to provide a sound foundation for European policies and legislation and to support the European Commission (EC), European Parliament and EU Member States in taking effective and timely risk management decisions. Within EFSA, the Panel on Contaminants in the Food Chain (CONTAM) provides scientific advice on contaminants in the food chain and undesirable substances.

Between 2010 and 2012 EFSA published its first assessments of the risks to human health related to the presence of brominated flame retardants (BFRs) in food, including: (i) polybrominated biphenyls (PBBs), (ii) hexabromocyclododecanes (HBCDDs), (iii) polybrominated diphenyl ethers (PBDEs), (iv) tetrabromobisphenol A (TBBPA) and its derivatives, (v) brominated phenols and their derivatives, and (vi) emerging and novel BFRs (https://www.efsa.europa.eu/en/topics/topic/brominated-flame-retardants). In 2018, the European Commission requested EFSA to update its previous assessments (except the one on PBBs) considering the occurrence data in food submitted to EFSA after the publication of the previous assessments, as well as the newly available scientific information of relevance to hazard identification and characterisation. In addition, the similarities in chemical properties and effects seen in the previous EFSA assessments for the different BFR families warrant the consideration of a mixture approach. The appropriateness of applying a mixture approach will be evaluated once the risk assessments for each individual BFR family have been updated, and will be based on the Guidance of EFSA for combined exposure to chemical mixtures (EFSA Scientific Committee, 2019).

The update of the risk assessment on HBCDDs was finalised and published in March 2021 (EFSA CONTAM Panel, 2021), while the update of the PBDEs in food assessment is on-going and will be published in 2023. The preparation of the updates of the opinions on TBBPA and other brominated phenols has started.

In this paper the methodology used to perform the updates of the risk assessments will be laid out, as well as the outcome of the updates of the risk assessment on HBCDDs and PBDEs in food.

2. Materials and Methods:
EFSA performs its risk assessments on contaminants following the general principles described by WHO/IPCS (2009) and the relevant EFSA guidance documents, and according to the risk assessment paradigm that includes hazard identification, exposure assessment, hazard characterisation and risk characterisation.

The exposure assessment combines the data on human consumption for the different food categories with the occurrence data on BFRs in the respective food categories. Estimates of exposure were provided for different age groups of the population. The occurrence data submitted to EFSA by European countries were used, including those generated following Commission Recommendation 2014/118/EU on the monitoring of BFRs in food (EC, 2014). Regarding the food consumption data, the EFSA Comprehensive European Food Consumption Database was used. This database provides a compilation of existing information on food consumption at individual level for different age groups across the European Union. All food consumption data were codified according to the FoodEx classification system which has been developed by the EFSA.

For the hazard characterisation, existing toxicological and toxicokinetics studies, including those from the open literature, were considered.

3. Results:
Update of the risk assessment of HBCDDs in food:
Exposure assessment: A total of 6,352 analytical results for HBCDDs in food submitted to EFSA fulfilled the quality criteria applied and were selected and used for the chronic exposure assessment. Contrary to the data used in the previous Opinion on HBCDDs published in 2011, where most of the data were analysed with GC-MS and reported as ‘Total HBCDDs’, the current data have mostly been analysed by LC-MS and reported on the specific stereoisomers α-, β- and γ-HBCDD. The highest mean concentrations of the sum of these three stereoisomers were found in the food category ‘Fish meat’. The mean dietary exposure...
estimates ranged from 0.07 (minimum lower bound (LB))/0.17 (minimum upper bound (UB)) to 0.79 (maximum LB)/1.52 (maximum UB) ng/kg bw per day across dietary surveys and age groups. At the 95th percentile, dietary exposure estimates ranged from 0.23 (minimum LB)/0.45 (minimum UB) to 2.30 (maximum LB)/3.61 (maximum UB) ng/kg bw per day. The high proportion of left-censored data, partially due to relatively high limits of quantification (LOQs), resulted in a two- to threefold difference between the LB and UB exposure estimates. The most important contributors to the chronic dietary LB exposure to HBCDDs were ‘Fish meat’, ‘Eggs’, ‘Livestock meat’ and ‘Poultry’. The Panel also noted that non-dietary exposure through intake of dust and dermal contact can substantially contribute, and in some cases even dominate the total human exposure to HBCDDs, especially for toddlers. An exposure scenario for breastfed infants was also estimated: the scenario based on average human milk consumption and the reported UB range for HBCDDs in pooled human milk samples collected in European countries between 2014 and 2016 as part of the WHO/UNEP field studies, resulted in a median exposure of 14.3 ng/kg bw per day, while the scenario based on high human milk consumption, resulted in median exposure of 21.5 ng/kg bw per day.

Hazard identification and characterisation: Based on the toxicological information from the previous Opinion and on new studies that became available since then, the CONTAM Panel concluded that the main targets for toxicity are neurodevelopment, the liver, thyroid hormone homeostasis and the reproductive and immune systems. A growing number of epidemiological publications were identified assessing the association between exposure to HBCDDs and birth weight/length, neurodevelopment and thyroid dysfunctions in children, as well as subfertility, type 2 diabetes, thyroid hormone levels, severe endometriosis (including ovarian endometrioma) and breast cancer metastasis in adults. None of the effects studied in longitudinal studies and using internal exposure measures either reached statistical significance or were replicated. Considerable limitations exist pertaining to small sample sizes, varying methodological quality, effect inconsistency and considerable heterogeneity in the assessed populations, exposures and endpoints. Adverse effects of HBCDDs related to neurodevelopment have been assessed in two epidemiological studies; the low volume of prospective data, the differing endpoint measures and the lack of replication render these data insufficient for use in hazard characterisation. Since the evidence from the available human data was not sufficient to base the risk assessment on, the data from studies on experimental animals were used to identify a Reference Point for the human health hazard characterisation. The CONTAM Panel concluded that the neurodevelopmental effects on behaviour can be considered the critical effect for the hazard characterisation of HBCDDs, and identified from a single dose study a LOAEL of 0.75 mg/kg bw for spontaneous behaviour (horizontal locomotion, rearing and total activity), corresponding to a body burden of 0.75 mg/kg bw at the LOAEL. The chronic intake that would lead to the same body burden in humans was calculated assuming an absorption in humans of 100% and the longest half-life identified in humans for HBCDDs of 219 days. This resulted in an estimated chronic human dietary intake of 2.35 µg/kg bw per day. Due to limitations in the database, the derivation of a health-based guidance value was not considered appropriate, and instead, the margin of exposure (MOE) approach was applied to assess possible health concerns. The CONTAM Panel considered that an MOE higher than 24 would indicate a low health concern, since this MOE would be sufficient to cover interspecies and intraspecies differences in dynamics (a factor of 2.5 and 3.2, respectively), as well as extrapolation from a LOAEL to a NOAEL (a factor of 3).

Risk characterisation: MOEs were calculated by comparison of the calculated chronic human dietary intake of 2.35 µg/kg bw per day, leading to the body burden at the LOAEL, with the estimated dietary exposure for the different population groups. The MOE values obtained ranged from 34,000 to 650. These MOEs are larger than 24 and the CONTAM Panel concluded that they do not raise a health concern. The CONTAM Panel also compared the body burden of 0.75 mg/kg bw at the LOAEL with the body burdens estimated in adults based on levels in adipose tissue, blood and milk reported in the literature. The results support the conclusion that current dietary exposure to HBCDDs in European countries does not raise a health concern. For breastfed infants, the lowest MOE values for high milk consumption are below the value of 24. The CONTAM Panel concluded that these MOEs may raise a health concern for some breastfed infants.

Update of the risk assessment of PBDEs in food:

The update of the risk assessment of PBDEs in food is on-going with a public consultation of the published draft assessment planned before its final adoption by the CONTAM Panel. In the previous Opinion (EFSA CONTAM Panel, 2011), eight congeners were considered to be of primary interest for dietary PBDE exposure: BDE-28, -47, -99, -100, -153, -154, -183 and -209. This was based on the composition of the technical PBDE products, occurrence in the environment and in food. In the current update, the Panel has focused on these eight congeners plus BDE-49 and -138 that were included in Commission Recommendation 2014/118/EU. The Panel has evaluated the occurrence data on these congeners in food submitted to EFSA, and the newly toxicological data available including those published in the open literature.
In the previous Opinion it was concluded that the main targets in sub-chronic and chronic toxicity studies in rats and mice for a variety of PBDE congeners and PBDE technical products were the liver, as the thyroid hormone, nervous, reproductive and immune systems. Relevant toxicity data were only available for BDE-47, -99, -153 and -209, and therefore a risk assessment could only be carried out for these four individual PBDE congeners. Effects on neurodevelopment, which affect behaviour in mice were identified as the critical endpoint, and BMDL_{10} (lower 95% confidence limit for a benchmark response of 10%) values were derived from studies using a single oral dose. Body burdens at the BMDL_{10} and its corresponding chronic human dietary intake were estimated. Due to the limitations and uncertainties in the current toxicological database on PBDEs, the derivation of a health-based guidance value was not appropriate. Therefore, a MOE approach was used for the risk characterisation. In the previous Opinion the Panel concluded that for BDE-47, -153 and -209 the MOEs do not indicate a health concern, while the MOEs for BDE-99 support the conclusion for a potential health concern.

In the current update, the new toxicological data were assessed, and the possibility of a combined risk assessment of congeners was considered in relation to the 2019 EFSA Guidance on combined exposure to chemical mixtures. The 2022 EFSA Guidance on benchmark dose modelling will also be applied.

Update of the risk assessment on the remaining classes of BFRs:
The update of the risk assessments on TBBPA and its derivatives in food has started. It will focus on TBBPA and the following derivatives: TBBPA bismethyl ether (TBBPA-bME, CAS No 70156-79-5), TBBPA bis(2-hydroxyethyl) ether (TBBPA-bOHEE, CAS No 4162-45-2), TBBPA bisallyl ether (TBBPA-bAE, CAS No 25327-89-3), Tetrabromobisphenol A bis(glycidyl ether) (TBBPA-bGE, CAS No 3072-84-2), and TBBPA bis(2,3-dibromopropyl)ether (TBBPA-bDiBPrE, CAS No 21850-44-2).

Regarding the update of the risk assessment on Brominated phenols and their derivatives in food, it will focus on 2,4,6-tribromophenol (2,4,6-TBP, CAS No 118-79-6), 2,4-dibromophenol (2,4-DBP, CAS No 615-58-7), 4-bromophenol (4-BP, CAS No 106-41-2), 2,6-dibromophenol (2,6-DBP, CAS No 608-33-3), tetrabrominated bisphenol S (TBBPS, CAS No 39635-79-5), and tetrabromobisphenol S bismethyl ether (TBBPS-BME, CAS No 70156-79-5). Both updates are expected to be finalised during 2024.

4. Discussion:
The on-going updates of the risk assessment on BFRs in food EFSA is undertaking provide insight into the current exposure estimates of BFRs via food compared to those estimates based on data collected over a decade ago. It should be noted that comparison of the current data with results from the previous Opinion is hampered by a number of facts, such as improvements in instrumental analysis, different percentage of left-censored data, consideration of further food commodities and use of more detailed consumption data. As an example, the age group 'Other children' was identified in the 2011 Opinion to have the highest dietary exposure to HBCDDs with mean and P95 dietary exposure ranging between 0.15–1.85 and 0.80–4.46 ng/kg bw per day, respectively. In the 2021 updated assessment, the respective estimated dietary exposures were somewhat lower, being 0.11–1.21 and 0.37–2.95 ng/kg bw per day, respectively. The same holds for dietary HBCDD exposure for adults. Regarding the food groups contributing most to the dietary exposure, in the previous 2011 Opinion, the contribution of fish meat to the median LB intake of HBCDDs across European dietary surveys varied from 83% to 88.2% for the different age groups. In the 2021 updated assessment, the contribution of fish meat to the LB chronic dietary exposure only ranged between 22% and 47.1%.

5. Conclusions:
EFSA is performing updates of its previous risk assessments on BFRs carried out over a decade ago. Once these updates are finalised, European and national decision-makers can use EFSA’s scientific advice as well as other considerations in weighing up any possible measures to reduce consumer exposure to BFRs in food. EFSA has no direct role in deciding such measures.

6. Acknowledgments:
EFSA wishes to thank the members of the EFSA WG on BFRs (Diane Benford, Laurent Bodin, Peter Fürst, Andy Hart, Christer Hogstrand, Evangelia Ntzani, Martin Rose, Henri Schroeder, Martine Vrijheid and Christiane Vlemmix), the members of the CONTAM Panel (https://www.efsa.europa.eu/en/science/scientific-committee-and-panels/contam) and the EFSA staff and collaborators Sofia Ioannidou, Marina Nikolić, Bruno Dujardin, Francesca Riolo, Elena Rovesti, Kelly Niermans and Federico Cruciani, that contributed to the development of the risk assessments. EFSA wishes to acknowledge all the European countries that provided BFR occurrence data in food and support the consumption data collection for the Comprehensive European Food Consumption Database.
7. References:
TUE-PM2-C3  Relative Potency Factors for Risk Assessment of Per- and Polyfluoroalkyl Substance Mixtures

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**Introduction:** Per- and polyfluoroalkyl substances (PFASs) often occur together as contamination in exposure media such as drinking water and food. In addition, PFASs exert common toxicological effects, which warrants cumulative risk assessment of these substances. The relative potency factor (RPF) approach is one of the methods facilitating risk assessment of mixtures which are dose-additive (van den Berg et al. 2006; van den Brand et al. 2022). In this research we derived RPFs for several PFASs to facilitate mixture risk assessment (Bil et al. 2021; RIVM 2021; 2023).

**Materials and Methods:** A database of liver endpoints was established from public literature for 17 PFASs, using data with the same species (rat), sex (male), and exposure route (oral) and comparable exposure duration. Dose–response analysis was applied to obtain the relative potencies of 3 perfluoroalkyl sulfonic acids, 9 perfluoroalkyl carboxylic acids, 2 perfluoroalkyl ether carboxylic acids, and 2 fluorotelomer alcohols compared to perfluorooctanoic acid (PFOA) (Figure 1). In addition, the RPFs of 7 other perfluoroalkyl acids were estimated based on read-across (not shown here). This resulted in the RPFs of 23 PFASs compared to the potency of index compound PFOA.

**Results:**

Figures 1: RPFs (black dots) and 90% confidence intervals for each PFAS. PFOA is the index compound (with RPF=1). Rounded values of the RPFs are listed at the left y-axis. Full names of the PFASs are listed on the right y-axis.

**Conclusion:** The obtained RPFs can be applied to PFAS concentrations in e.g. drinking water or food, or to human exposures, resulting in the sum of PFOA equivalents in a mixture. This sum can be compared with an established PFOA concentration limit or an external health-based guidance value (e.g., tolerable daily intake or reference dose) to estimate the risk resulting from direct oral exposure to PFAS mixtures. Assessing mixture exposure is particularly relevant for PFASs, with omnipresent exposure in our daily lives.

**References:**
1. Bil et al. (2021) https://doi.org/10.1002/etc.4835
2. van den Brand et al. (2022) https://doi.org/10.3390/toxins14050303
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Introduction: Recent epidemiological studies show that current levels of exposure to PCBs remain of great concern, as there is still a link between such exposures and the development of chronic environmental diseases. While most studies have focused on the health effects caused by exposure to dioxin-like PCBs (DL-PCBs), chemical exposure to non-dioxin-like PCB (NDL-PCB) congeners, although significant, is less investigated. In addition, adverse effects of PCBs have been documented in humans after accidental and massive exposure, but little is known about the effect of chronic exposure to low-dose PCB mixtures. Omics approaches have recently emerged as interesting alternative methodologies to address the risk assessment of chemicals and involve a shift in the way toxicological studies are conducted. The objective of this study was to identify biomarkers of effect associated with NDL-PCB exposure at dietary exposure levels. Pig was selected as animal model due to the similarities with human metabolism.

Materials and Methods: Six female pigs were randomly assigned to control and exposed groups. Exposed pigs received orally a daily dose of Aroclor 1260 (20 ng/kg b.w.) in sunflower oil. Aroclor 1260 contains 30.7% by weight of the 6 indicator PCBs. The exposure dose selected (6.1 ng/kg b.w. per day 6 NDL-PCBs) was based on the observed P95 exposure level of the French population in the second Total Diet Study (i.e. 7.9 ng/kg b.w. per day). This exposure level is also close to but slightly lower than the associated tolerable daily intake (i.e. 10 ng/kg b.w. per day). Serum samples were regularly collected over a month. The metabolomics and lipidomics studies involved the use of liquid chromatography (both RP and HILIC) and gas chromatography coupled to high resolution mass spectrometry (LC-HRMS and GC-HRMS) methods.

Results: In the LC-metabolomics analysis, 33 metabolites have been identified as significantly altered by the Aroclor administration, while in the LC-lipidomics analysis, 39 metabolites were putatively annotated and associated with NDL-PCB exposure. In the GC-metabolomics analysis, the serum profiles of 84 metabolites was observed as significantly altered by the administration of Aroclor 1260. By combining the three data sets, the consequences of exposure to chronic doses of Aroclor1260 could be detected in the metabolism of pigs. Changes were observed in the metabolic pathways of: 1) fatty acids (especially unsaturated fatty acids), 2) glycolysis and the pentose-phosphate pathway, 3) Krebs cycle, 4) neurotransmitters (GABA, serotonin, kynurenine, etc.), 5) purine metabolism and 6) gut microbiota derived metabolites (indole acids, and some secondary bile acids).

Discussion and Conclusion: Combining three analytical platforms allowed complementary investigations of metabolism disruptions. The aggregate interpretation of the results of this study provides a substantial and concise overview of the effect of low dose exposure to NDL-PCBs, reflecting the hepatotoxic and neurotoxic effects already reported in literature at higher and longer exposures. The main hepatotoxic biomarkers observed in this study were the altered arachidonic acid and linoleic acid metabolism, together with the altered bile acids, indole and serotonin production driven by the gut microbiome. On the other end, the main neurotoxic biomarkers could be observed in the enhanced glycolysis, the disrupted Krebs’ cycle, and the changes in the concentration of several neurotransmitters.

Acknowledgments: European Union’s Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement HAZARDoomics No 795946. Spanish grant PID2020-120020RA-I00 funded by MCIN/ AEI/10.13039/501100011033.

References:
TUE-PM2-D2 Chlorinated value organophosphate esters in Irish waste foams and fabrics: concentrations and evaluation of the impact of a concentration limit

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Introduction: To meet flame retardancy regulations, chlorinated OPEs (i.e., tris(2-chloroethyl) phosphate (TCEP), tris(chloropropyl) phosphate (TCIPP) and tris(1,3-dichloro-2-propyl) phosphate (TDCIPP)) have found extensive application. Given Cl-OPEs are entering new articles unintentionally via recycling, combined with the proposal of the European Chemicals Agency (ECHA) to prohibit some applications of Cl-OPEs, a limit similar to that existing for concentrations of some brominated flame retardants (BFRs) may in future be placed on concentrations of TCEP, TCIPP, and TDCIPP in waste. This work tests the hypothesis that Cl-OPE use in Ireland has been extensive resulting in a high proportion of waste containing elevated concentrations of Cl-OPEs.

Materials and Methods: To test our hypothesis, we measured concentrations of Cl-OPEs in 273 samples of waste comprising: construction and demolition (C&D) insulation foam (expanded and extruded polystyrene (XPS and EPS)), as well as foam and fabrics from end-of-life vehicles (ELV), and domestic soft furnishings. Samples were collected between 2019 and 2020 from waste handling sites in Co. Galway, Ireland. On receipt at Birmingham, samples were analysed for Cl-OPEs via solvent extraction followed by GC-MS.

Results: Out of the 273 samples, 82 (30%) contained at least one Cl-OPE at >1,000 mg/kg, which at the time of sample collection was the limit placed on concentrations of some BFRs within the EU. Exceedances of the notional 1,000 mg/kg limit occurred mainly for TCIPP and TDCIPP. TCIPP exceeded 1,000 mg/kg in 8/13 (62%) C&D XPS insulation, 18/49 (37%) ELV foam, and in 6/16 (38%) furniture foam samples. By comparison, concentrations of TDCIPP >1,000 mg/kg in 2/12 (17%) C&D EPS insulation, 21/49 (43%) ELV foam, and 3/16 (19%) furniture foam samples. Table 1 shows the mass of different waste categories generated in 2019 in Ireland and the mass of Cl-OPEs in that waste. It also depicts the mass of waste containing one or more Cl-OPEs at >1,000 mg/kg and the amount of Cl-OPEs removed if such waste were diverted from the recycling stream and treated as hazardous.

Table 1: Estimated mass of waste generated in Ireland in 2019 and associated mass of Cl-OPEs

<table>
<thead>
<tr>
<th>Category</th>
<th>t waste/yr</th>
<th>t waste &gt;1,000 mg/kg/yr</th>
<th>∑Cl-OPEs in waste (kg/yr)</th>
<th>∑Cl-OPEs in waste &gt;1,000 mg/kg (kg/yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C&amp;D insulation foam</td>
<td>5,500</td>
<td>2,200</td>
<td>22,000</td>
<td>22,000</td>
</tr>
<tr>
<td>ELV foams/fabrics</td>
<td>3,800</td>
<td>1,600</td>
<td>66,000</td>
<td>65,000</td>
</tr>
<tr>
<td>Carpets</td>
<td>7,600</td>
<td>380</td>
<td>930</td>
<td>610</td>
</tr>
<tr>
<td>Curtains</td>
<td>740</td>
<td>59</td>
<td>200</td>
<td>120</td>
</tr>
<tr>
<td>Furniture foam</td>
<td>2,600</td>
<td>1,300</td>
<td>31,000</td>
<td>31,000</td>
</tr>
<tr>
<td>Furniture fabrics</td>
<td>880</td>
<td>170</td>
<td>810</td>
<td>480</td>
</tr>
<tr>
<td>Mattress foam</td>
<td>6,100</td>
<td>1,100</td>
<td>24,000</td>
<td>24,000</td>
</tr>
<tr>
<td>Mattress fabrics</td>
<td>2,500</td>
<td>380</td>
<td>1,700</td>
<td>720</td>
</tr>
<tr>
<td>Total</td>
<td>30,000</td>
<td>7,200</td>
<td>147,000</td>
<td>144,000</td>
</tr>
</tbody>
</table>

Discussion and Conclusion: Enforcement of a 1,000 mg/kg limit on each of TCEP, TCIPP, and TDCIPP in waste, such that items exceeding this value were not permitted to be recycled, would result in removal of 144,000 kg/yr out 147,000 kg/yr (~98%) of the sum of these Cl-OPEs from the Irish recycling stream. While this would also result in ~7,200 t/yr of such waste (24% of the ~30,000 t/yr generated in 2019) being rendered unrecyclable; the effectiveness of such a limit in removing Cl-OPEs from the recycling stream is clear.

Acknowledgement: This project (SAFER, reference 2018-RE-LS-3) is funded under the EPA Research Programme 2014-2020. The EPA Research Programme is a Government of Ireland initiative funded by the Department of Communications, Climate Action, and Environment.
Introduction: In Japan, the collection and recycling of waste electrical and electronic equipment (WEEE) are regulated by two recycling laws: the Home Appliance Recycling Law and the Act on Promotion of Recycling of Small WEEE (sWEEE). The Home Appliance Recycling Law specifically applies to four categories of post-consumer home appliances: air conditioners, refrigerators/freezers, washing machines/clothes dryers, and televisions. According to this law, manufacturers or importers are responsible for the collection and recycling of home appliances within these four categories. WEEE items outside these four categories are regulated by the Law on Promotion of Recycling of sWEEE, but their collection and recycling are not mandatory. While it is common to recover only metals from sWEEE-derived waste through heat treatment or nonferrous metal smelting, some companies have started implementing plastic sorting technologies, such as sink/float separation, to recover plastics as secondary raw materials for recycling. However, there is a lack of research regarding contamination with regulated plastic additives, such as polybrominated diphenyl ethers (PBDEs), which are listed as persistent organic pollutants (POPs) under the Stockholm Convention. In order to obtain basic information to facilitate the recycling of such plastics, in this study, we analyzed the composition of mixed plastic waste derived from sWEEE; screened bromine (Br) contents as an indicator of brominated flame retardants (BFRs); and measured the actual content of PBDEs.

Materials and Methods: In November 2020, mixed plastic waste samples resulting from shredding sWEEE and subsequent metal removal in the recycling process were collected at a designated recycler in Japan. Five lots of flake samples (A to E) were obtained, each weighing approximately 20 kg. The samples were divided into three particle sizes by sieving them through 5 mm and 20 mm sieves. The composition of the 5−20 mm and >20 mm fractions were examined, and the flakes were classified as hard plastics, soft plastics, urethane foam and other lightweight materials, metals, copper wire, printed circuit boards, printed wire, rubbers, wire coating, wood, paper, or glass. The >20 mm fractions of hard plastics were separated by color, and the Br content of each flake was individually screened using a handheld X-ray fluorescence analyzer (XRF, Olympus DELTA, analytical time set to 30 s in RoHS/WEEE mode) as an indicator of PBDE content. For flakes with Br contents >1,000 mg/kg, total reflection absorption Fourier transform infrared spectroscopy (ATR-FTIR, Agilent 5500 Compact FTIR) was employed to further assess PBDE occurrence and to determine the plastic type by polymer.

Results: The weight percentage of mixed plastics derived from sWEEE did not differ significantly among the lots, with approximately 70% of the total distributed in the 5−20 mm fractions. Compositional analysis of the 5−20 mm and >20 mm fractions, which accounted for most of the weight fraction, revealed that approximately 65% of both fractions consisted of hard plastic. This finding suggests that the composition of mixed plastics after metal removal is similar. XRF screening revealed that about 80−90% of the hard plastic in the >20 mm fraction was free of BFRs. Plastic flakes containing >1% Br accounted to 5−10% of the total, but only 0.3% of the total was identified as containing PBDEs. This indicates that most plastic flakes with high Br content were treated with unregulated BFRs other than PBDEs. Based on the results of this study, the estimated Br contents in the shredded sWEEE plastics ranged from 4,500 to 6,600 mg/kg, with a corresponding PBDE concentration of approximately 300 mg/kg. Moreover, PBDE-derived Br accounted for only about 5% of the total Br content.

Discussion and Conclusion: Even when the Br content in sWEEE mixed plastics is high, it is unlikely for the PBDE concentration to exceed the low POP content (LPC) limits of 500 mg/kg or 1,000 mg/kg proposed under the Basel Convention. Therefore, if the presence of regulated BFRs is solely determined based on the Br content, most mixed plastics would be misclassified as non-recyclable. It is imperative to accumulate evidence showcasing that PBDE concentrations are sufficiently low in specific recycling streams, based on measurement data like in this study, for promoting plastic recycling regardless of the total Br content.

Acknowledgments: This study was conducted in cooperation with Re-Tem Corporation, Japan. This study was financially supported by the Environment Research and Technology Development Fund [grant numbers JPMEEEEF20193301, JPMEEERF20233301] of the Environmental Restoration and Conservation Agency provided by Ministry of the Environment of Japan.
**TUE-PM2-D4 An Introduction to and Achievements of the GEF Project: “Improvement of the Environmental Performance of the Foam Sector: Phase out and Management of Hexabromocyclododecane in China”**

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**Introduction:** Hexabromocyclododecane (HBCD) was one of the main brominated flame retardants produced since the 1960s with a total historic production of 750,000 t[1]. More than 90% of HBCD has been used in EPS/XPS foam for insulation in housing and construction. Considering an average use of 1.5% this translates to a global past production of 45 million tonnes of HBCD containing EPS/XPS which due to the long service life of 30 to 50 years and longer is largely still present in buildings[2]. HBCD has been listed in the Stockholm Convention in 2013 with an exemption for production and use in in EPS/XPS foam for insulation foam. China was the last producer of HBCD with a yearly production of more than 18,000 tonnes by several producers.[3]

**Materials and Methods:** In order to promote the elimination of HBCD in China, the Foreign Environmental Cooperation Center of the Ministry of Ecology and Environment (FECO-MEE) and the United Nations Industrial Development Organization (UNIDO) developed the Global Environment Facility (GEF) project "Improvement of the Environmental Performance of the Foam Sector: Phase-out and Management of HBCD in China".

**Results:** Cooperating with GEF and UNIDO, with the support of the HBCD project, China as last producing country achieved the total phase-out of HBCD from all producers, before the exemption expiration date on December 25th, 2021. Under the project framework, Shandong province where the HBCD production in China was majorly located is the demonstration province for HBCD phase-out, HBCD management and alternatives. Three demonstration projects on the halt and conversion of HBCD production have been developed resulting in the stop of HBCD production. This contributed to an annual HBCD elimination of over 18,000 tonnes. Five demonstration projects on HBCD alternatives in EPS and XPS industries have been developed, promoting the application of HBCD alternatives such as brominated-SBS and TBBPA-BDBPE (methyl-octabromo-ether) in the foam industry. The project has performed research on HBCD management, analysis on industrial policies in the foam industry. Based on the research, analysis and experiments, standards and guides on HBCD waste management and treatment have been drafted. The project is also concerned about and has conducted subprojects on gender mainstreaming in the foam industry as well as publicity and public education on HBCD prohibition.

**Discussion and Conclusion:** China commits itself to fulfilling the requirements of the Stockholm Convention. The HBCD project with its phase-out of the last HBCD production and improves the environmental performance of the foam industry in China, reducing environmental contaminating and protecting people’s well-being. Due to the long service life of 50 years and longer, most of the large HBCD containing EPS/XPS is still present and will need to be managed in the decades to come. The current project has a component on inventory of HBCD containing EPS/XPS and managing HBCD containing waste including demonstration projects for environmentally sound destruction of HBCD waste by municipal solid waste incineration technology, hazardous waste incineration technology, and co-processing in cement kilns. Therefore through this UNIDO/GEF project China will gain experience in managing and destroying HBCD containing waste and will develop and document relevant experience to make contributions to global environmental governance and waste management of HBCD containing EPS/XPS which can be utilized by other countries including developing economies.

**Acknowledgments:** Funding from the Global Environmental Facility and from project partners are acknowledged

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Exploring legacy and emerging PFAS in songbird eggs collected near a fluorochemical factory in Flanders using target analysis, suspect screening and Total Oxidizable Precursor Assay (TOPA)

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Introduction: Large-scale mapping project revealed the presence of PFAS at high levels at thousands of sites across Europe and the region of Flanders was identified as one of the main hotspots due to the presence of the 3M fluorochemical factory near Antwerp and other facilities, such as firefighting training sites [1]. Previous studies near this hotspot revealed some of the highest concentrations ever reported in soils, invertebrates and songbird eggs, blood plasma and feathers [2,3,4]. However, these studies focused on legacy PFAS, like PFOS and PFOA, analysed with a target approach. As bird eggs have proven to be a useful biomonitoring tool for the exposure assessment of PFAS-contaminated areas, the main objective of this work was to combine target analysis with suspect screening and the Total Oxidizable Precursor Assay (TOPA), in order to study the bioaccumulation of emerging PFAS in bird eggs.

Materials and Methods: In total, 29 great tit (Parus major) eggs and 10 blue tit (Cyanistes caeruleus) eggs were collected at three different sites within a 0-1.5 km radius of the 3M plant in April-May 2022. Solid-phase extraction with WAX cartridges was used to extract 0.2 grams of homogenized egg sample [2]. UHPLC-MSMS analysis was performed for the target analysis of 26 PFAS, and LC-QTOF analysis for the suspect screening. Two suspect lists - for a total of >10000 PFAS - were used to identify suspect features. For the TOPA, seven PFAS precursors identified in the most contaminated eggs (n=6), were selected as model compounds to be used for the method optimization that will be carried out in the coming period.

Results: Target analysis showed that PFOS was predominant in the eggs, with concentrations ranging from 14 to 67920 mg/kg ww (average=6780 mg/kg ww). Long-chain PFCAs and PFSAs were primarily detected in the eggs and PFHxS, PFHpS and PFDS were only detected in eggs collected within a 1 km radius of 3M. Preliminary results of the suspect screening showed the presence of precursors in the most contaminated samples, like the fluorotelomer sulfonate 6:2 FTS, and perfluorooctanesulfonamides such as FOSA and Et-FOSA.

Discussion and Conclusion: The PFAS composition profile found in great and blue tit eggs is comparable to previous studies conducted in 2011 and 2015 on the same species and sampling sites [4,5]. Although PFOS was phased out in 2006, it is still the predominant PFAS, with concentrations that are still among the highest ever reported in wild bird eggs. The presence of PFOS can be due to its persistence and bioaccumulation through the diet, and/or in vivo biotransformation of some precursors, such as those detected in this study, e.g. FOSA. The use of TOPA can highlight the presence of precursors and, to our knowledge, this is the first study to apply this method to passerine bird eggs. Since TOPA results are expressed as an increase or decrease in the concentration of perfluoroalkyl acids after oxidation, the combination with suspect screening can help identify precursors present in the samples and compounds not affected by oxidation (e.g. GenX). The alternative compound cC6O4 has already been measured in great tits eggs [6], pointing out the urgency of broadening PFAS characterisation using multiple approaches.

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**WED-AM-A2  Perfluoroalkyl acids in shorebird, shellfish, and sediment after total oxidizable precursor assay**

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**Introduction:** The total oxidizable precursor (TOP) assay as a promising technique can integrate the unknown or undetected precursors to perfluoroalkyl acids (PFAAs)¹. This technique has been successfully applied to wastewater, soil, textile, human plasma, firefighting foams, chicken eggs, etc². However, data about changes of PFAA in biota samples after TOP assay are still scarce. In this study, we applied TOP assay to the liver from shorebirds, shorebird blood cells and serum, shellfish (the food of the shorebirds) and sediment collected from Australia and China.

**Materials and Methods:** Per- and polyfluoroalkyl substances (PFASs) in liver (25), blood cells and serum (27), and shellfish (30) were extracted by MeOH with 1% w/v ammonium formate and purified by HybridSPE. PFASs in sediment (27) were extracted by a mixture of MeOH and water and purified by HLB SPE. Part of the extracts from all samples were oxidized by Na₂S₂O₈ and NaOH at 85°C for 24 h, then neutralized by HCl and the oxidant products were extracted by MTBE. Final extracts before and after TOP assay were analyzed by UPLC-MS/MS.

**Results:** The average ∆PFCAs after TOP assay in bird liver, blood cell and serum, shellfish, and sediment were 3263, 46, 7.6, and 2.3 ng/g (w.w.), respectively. Comparing the concentration of PFCAs in these samples before TOP assay, the increase in concentrations were 65878%, 335%, 243%, and 1012% in bird liver, blood cell and serum, shellfish, and sediment. The distribution of the individual ∆PFCA after TOPA in these four types of samples is shown in Figure 1.

**Discussion:** The observed ∆PFCAs was highest in bird samples (liver, blood cell and serum), following shellfish, and sediment, indicating a potential accumulation of the PFAA precursors in the food chain. In addition, the highest increases of PFCAs were found in bird liver which is the main detoxifying organ in birds, indicating the huge amount of PFAA precursors in bird liver. The average yield of PFCAs in bird liver was even higher than that in a previous study which reported the average increase of PFCAs was 11150% in soil collected from an aqueous film forming foam factory area¹. This indicates that current monitoring studies on PFASs are a large underestimation (as only the tip of the iceberg) and do not account for unknown PFAA precursors, especially in bird liver.

**References:**
Per- and Polyfluorinated Substances (PFAS): Occurrence and Exposure

Chairs: T. Groffen & Y. Yao

Luise Zimmermann¹, René Lämmer¹, Bernd Göckener*¹, Mark Bücking¹, Gábor-Árpád Czirják²

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**Introduction:** Per- and polyfluoroalkyl substances (PFAS) are a group of anthropogenic chemicals with several industrial and commercial application uses. PFAS entering the environment are globally distributed through atmospheric and oceanic currents through which they can reach remote regions of the Earth. The arctic fox (*Vulpes lagopus*) is native to Iceland and is therefore an example for apex predators in remote regions. Although studies on the presence of PFAS in arctic foxes exist, relatively little is known about correlations between PFAS contamination and body conditions.

**Materials and Methods:** In this study, the PFAS concentrations of several tissues (liver, kidney and muscle tissue) of artic foxes from Iceland were determined. All samples were extracted by either MTBE (liver and kidney) or acetonitrile (muscle) and measured by UHPLC-HRMS. Experimental data was then correlated with individual metadata of the arctic foxes (sex, age, habitat, etc.).

**Results:** Overall, 26 PFAS were identified in the fox matrices. Most quantified substances were long-chain PFCAs and PFSAs (C₆ to C₁₄) with PFOS being the most abundant. PFAS concentrations were the highest in liver samples, followed by kidney samples. Muscle tissue showed the lowest PFAS levels. Arctic foxes with habitats close to the coast showed higher ∑PFCAs (factor 10) and higher ∑PFSAs (factor 40) concentrations compared to foxes living inland. Females showed higher ∑PFCAs concentrations compared to males, although without statistical significance. Juvenile foxes displayed lower PFAS concentrations compared to adult animals.

**Discussion and Conclusion:** Several influencing factors on the PFAS burden of Icelandic artic foxes were identified. Differences between tissue types can be related to the function of the organs and the increased binding affinity of PFAS in high-protein tissues. The marine diet and biomagnification of PFAS in the marine food web support higher PFAS amounts found in artic foxes living in coastal areas of Iceland. Additional elimination pathways (e.g. gestation, lactation) may not play a significant role in the elimination of PFAS in female arctic foxes. A long half-life of PFAS in the body due to enterohepatic recirculation may lead to higher PFAS burdens with increasing age. Further investigation of PFAS in animals living in remote areas need to be carried out to deepen the knowledge about influencing factors of PFAS distribution in wildlife.
Per- and Polyfluorinated Substances (PFAS): Occurrence and Exposure

Chairs: T.Groffen & Y.Yao

WED-AM-A4  Muscle and liver distribution of perfluoroalkyl substances (PFASs) in freshwater species from Lake Trasimeno (central Italy)

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Introduction: Perfluoroalkyl substances (PFASs) are fluorinated aliphatic compounds widely used in various industrial products and processes [1]. They are environmental pollutants and due to their persistence and bioaccumulative nature, threaten biota and humans [2]. In January 2023, maximum levels for PFASs (PFOS, PFOA, PFNA, PFHxS and their sum) in food were established [3]. Studying aquatic organisms is particularly important because they can accumulate contaminants from water. This research focuses on assessing PFAS concentrations in fish species from Lake Trasimeno, Italy, and investigating their distribution in fish tissues. The aim is to understand the mechanisms of PFAS accumulation, evaluate environmental pollution levels, and ensure food safety.

Materials and Methods: A total of 19 linear PFASs (PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFteDA, L-PFBS, L-PFPeS, L-PFHxS, L-PFHpS, L-PFOS, L-PFNS, L-PFDS, and L-PFDoDS) and 2 branched PFASs (br-PFOS and br-PFHxS) were analyzed using LC-MS/MS, as previously described [4]. The muscle and liver tissues of four species from Lake Trasimeno were investigated: C. carassius (n=7), P. fluviatilis (n=8), A. anguilla (n=9), and the crayfish P. clarkii (n=9; only muscle).

Results: In the muscle tissue, the highest concentration of Σ19PFAS was found in A. anguilla (1.05±0.34 µg/kg), which had the highest fat content. C. auratus had the next highest levels (0.79±0.18 µg/kg), followed by P. fluviatilis (0.66±0.14 µg/kg), and P. clarkii (0.38±0.28 µg/kg). In liver samples from the same species, PFAS levels were 5, 8, and 7 times higher than in A. anguilla, P. fluviatilis, and C. auratus muscles, respectively. The highest concentration in the liver was found in C. auratus (5.73±2.72 µg/kg), followed by A. anguilla (5.33±1.78 µg/kg), and P. fluviatilis (5.23±1.27 µg/kg). The mean levels of Σ19PFAS in the liver were all very similar among the species, unlike the muscle tissue. None of the samples exceeded the maximum limits set for the sum of four PFASs in fish [3].

Discussion and Conclusion: In both muscle and liver of the fish species studied, PFOS was found to be the predominant analyte, followed by PFOA and PFNA in A. anguilla. In the other species, the prevalent analytes were long-chain carboxylic acids (C10-C14). Interestingly, in P. clarkii PFOS showed much lower levels, and C8-C14 carboxylic acids were predominantly detected. Other studies on freshwater fishes in Italy have been conducted, but very few have focused on the species selected in this monitoring. The results revealed lower levels of PFOS in the muscle of P. fluviatilis from Lake Trasimeno compared to that sampled in Lake Varese [5-6]. PFOS concentrations in A. anguilla liver and muscle were similar to those reported in the same species from the Po River and Laguna di Comacchio. Conversely, PFOA levels in the Trasimeno samples were much lower [7]. It is important to note that the data presented is very preliminary: additional samples and a more comprehensive statistical evaluation are required to obtain more detailed information.

Acknowledgments: RF-2019-12370587 (Financed by Italian Health Ministry)

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Introduction:

Per- and polyfluoroalkyl substances (PFASs) are a class of synthetic organofluorine substances with excellent biochemical stability and high surface activity. Oceans are believed to be one of their final global sinks. However, compared with legacy PFASs, studies on the bioaccumulation potential of emerging PFASs are still relatively limited. The Greater Bay Area (GBA) in China is one of the most developed regions in China, and the rapid industrialization and urbanization in this region have resulted in the substantial release of PFASs. Therefore, it is imperative to investigate the biomagnification characteristics of PFASs, particularly emerging PFASs, in the marine food web of this region, which also contribute to water quality management and marine conservation.

Materials and Methods:

Seawater ($n = 26$) and marine organisms ($n = 62$), including 21 crustacean species, 15 fish species, and two marine mammal species, were collected in the northern South China Sea in 2020. Target analysis and nontarget screening of PFASs were performed on these samples using ultra-performance liquid chromatograph interfaced with MS/MS and quadrupole time-of-flight MS, following our previous study.

Results:

Significant biomagnification was found for 22 legacy and emerging PFASs. A short-chain PFAS named perfluorooctanoic acid (PFHxA) was also found to have biomagnification potential. The trophic magnification factor (TMF) of $n$-PFOS was higher than those of branched isomers. Perfluoroethylcyclohexane sulfonate (PFECHS) was not detected in seawater and hardly observed in crustacean samples; however, it was widely found in biota samples. This study reported the difference in TMF between two PFECHS isomers, where trans-PFECHS had a slightly higher TMF than cis-PFECHS. TMFs of chlorinated polyfluorinated ether sulfonates were higher than that of perfluorooctane sulfonate (PFOS), indicating the higher biomagnification potential of these two emerging PFASs. The occurrence of bis[(trifluoromethyl)sulfonyl]imide (NTf2) in the seawater was revealed. One Cl-PFOS interfering substance with the proposed formula of $C_{14}H_{23}O_5SCl_6$ was identified. The daily intake of PFOS via seafood consumption for the local population was very close to the threshold.

Discussion and Conclusion:

The biomagnification potential of PFHxA indicated the contribution of PFAS precursor degradation. The detection of PFECHS in the biota but not in the seawater suggests its biomagnification potential. More investigation on the environmental behavior of Cl-PFOS is needed, and attention to the Cl-PFOS interference should be paid during the analysis of Cl-PFOS in complex matrices so as to avoid any false detection. It could be inferred that NTf2 might have a low bioaccumulation potential. These results suggest a requirement for further efforts to reduce the production and use of PFOS in the GBA to lessen the subsequent discharge and eventual deposit of these contaminants in marine ecosystems.

Acknowledgments:

The present work was supported by the National Key Research and Development Program of China (2022YFC3204800), Guangdong Basic and Applied Basic Research Foundation (2021A1515012048), Theme-Based Research Scheme of Research Grants Council of the Hong Kong SAR Government (T21-602/16-R), and Innovation Group Project of Southern Marine Science and Engineering Guangdong Laboratory (Zhuai) (311020004). This work was also supported by the Innovation and Technology Commission (ITC) of the Hong Kong SAR Government (9448002).

Reference:

**Introduction:** Per- and polyfluoroalkyl substances (PFASs) are a group of fluorinated organic chemicals applied for industrial and commercial products since the 1950s (Kissa et al., 2009; Lindstrom et al., 2011). PFASs are widely distributed through multiple environmental matrices, and their emissions into aquatic environments could pose a threat to health of aquatic organisms and consumers. In this study, 17 species, comprising 56 muscle, 6 livers, and 18 egg tissues, were sampled from three major rivers of Korea to understand the bioaccumulation and biomagnification processes of PFASs in freshwater food-webs. This study was first study on occurrence of PFASs and their precursors and alternatives in the Korean freshwater food web.

**Materials and Methods:** Thirty-two PFASs, including 17 PFAAs (PFBS, PFHxS, PFOS, PFDS, PFBA, PFDS, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTeDA, PFHxDA, and PFOcDA), 23 precursors, and intermediates (FOSA, N-MeFOSA, N-EtFOSA, N-MeFOSAA, N-EtFOSAA, 6:2 FTS, 8:2 FTS, 6:2 FTUCA, 8:2 FTUCA, 10:2 FTUCA, 6:2 diPAP, and 8:2 diPAP), and 3 alternatives (Gen-X, ADONA, and F-53B), in samples were measured using a liquid chromatography coupled to a tandem mass spectrometer as well as, and the ∼15N of amino acids to provide accurate trophic level (TL). 0.2 g dw of freeze-dried soft tissue was used for PFASs analysis. 5 ng of internal standards were spiked to sample and 1 mL of 0.5 M TBA (adjusted to pH 10) and 2 mL of 0.25 M Na2CO3 buffer were added for ion-pairing extraction. Oasis WAX cartridge was used for further clean-up procedure.

**Results:** Perfluorooctane sulfonic acid (PFOS) was a predominant compound, followed by C9–C13 carboxylic acids (PFCAs). The mean PFOS concentration was higher in adult liver tissue (253 ng/g dry wt), followed by eggs (79.6 ng/g dry wt) and muscle (32.6 ng/g dry wt). Among the 12 precursors, perfluorooctane sulfonamide (FOSA) was detected in all samples (mean: 9.33, 0.44, and 0.34 ng/g dry wt for liver, egg, and muscle, respectively). In this study, trophic magnification factor (TMF) was the highest for PFOS (3.44), followed by long-chained PFCAs (C9–C13; 1.49–4.81), and FOSA (1.42).

**Discussion and Conclusion:** PFASs in fish liver accumulated more than 1-5 orders of magnitude higher than those for fish muscle because PFASs better to bind with proteins in blood of liver (tissue selectivity). The relative concentration ratio of egg to muscle for PFASs ranged from 0.7 to 17.5. As carbon chain increases, the partitioning ratio increased suggests more transfer of long-chain carboxylates. For the field based TMF values, PFOS (mean: 3.4), and long chain PFCAs (2.2 for PFNA, 4.8 for PFDA, 4.5 for PFUnDA, 2.7 for PFDoDA, and 2.8 for PFTrDA) were biomagnified through food web in Korean freshwater ecosystem. This result consistent with previous studies that shorter-chain (C<8) PFCAs are not bioaccumulative or have limited potential bioaccumulation (Fang et al., 2014). Our findings suggest that several PFASs have a potential for bioaccumulation and biomagnification. The present study supports new insights on biomagnification of newly introduced PFASs for assessing risks in the freshwater food-web.

**Acknowledgments:** This work was supported by the Korea Environment Industry and Technology Institute (KEITI) through the Technology Development Project for Safety Management of Household Chemical Products Project funded by the Ministry of Environment (MOE) of Korea [2020002970007, 1485018715].

**References:**

WED-AM-B1  PCBs, pesticides, and lipids in the mixture analysis of the Anniston Cohort

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Introduction: About half of the total industrial production of the polychlorinated biphenyls (PCBs) in the United States occurred in a chemical plant in Anniston, Alabama (1935–1971). Elevated concentrations of PCBs have been previously reported in Anniston residents. Here we expand on a study of single exposure PCB associations with lipids using mixture analyses approaches.

Materials and Methods: Serum was collected from 338 participants and 35 ortho-substituted PCBs and chlorinated pesticides were analyzed at the National Center for Environmental Health using gas chromatography-isotope dilution high-resolution mass spectrometry (Sjödin et al., 2004). Lipids, including total cholesterol and triglycerides, were analyzed by the Northwestern Research Laboratory, Seattle, Washington. This analysis included 191 adults from the baseline Anniston Community Health Study I (ACHS I) who also participated in the follow-up, Anniston Community Health Study II (ACHS II). We have excluded 147 participants from this analysis that were taking medications which reduce lipids and cholesterol levels. A four-structure activity PCB groups ([non-ortho] mono-ortho, di-ortho and tri- and tetra-ortho substituted PCBs) were used for statistical analyses to reduce the number of correlated mixture components (van den Berg et al., 2006). Quantile g-computation was used to assess total mixture effects and relative contribution of individual exposures using the "qgcomp" R software package. All models were adjusted for age, sex, race (White, African American), body mass index, and history of ever smoking. Continuous measures of serum triglycerides and total cholesterol concentrations were analyzed.

Results: We observed statistically significant increases in overall marginal structural effects for total cholesterol ($\psi=15.45$, Confidence Interval 8.60–22.31, $p<0.0005$) and for triglycerides ($\psi=45.28$, Confidence Interval 25.35–65.212, $p<0.0005$) for each quantile change of the mixture containing four PCB groups, trans-nonachlor, and p,p'-dichlorodiphenyldichloroethylene (p,p'-DDE). Trans-nonachlor, non-ortho PCBs, p,p'-DDE, and tri- and tetra-ortho PCBs showed the highest relative positive weights for total cholesterol and triglycerides while di-ortho PCBs, and mono-ortho PCBs had negative weights.

Discussion and Conclusion: Higher total cholesterol and triglycerides are established risk factors for cardiometabolic health outcomes, including cardiovascular disease and hypertension. We observed statistically significant overall positive exposure mixture effects for total cholesterol and triglycerides in ACHS II. These observed effects suggest that both aryl-hydrocarbon receptor dependent (for dioxin-like non-ortho PCBs) and independent (for pesticides, tri- and tetra-ortho PCBs) mechanisms may be involved. These results suggest that increased exposure to some PCBs and pesticides may contribute to increases in total cholesterol which could contribute to negative cardiometabolic health outcomes.

Acknowledgements: We thank the study participants for taking part in the study. The findings and conclusions in this report are those of the author(s) and do not necessarily represent the views of the Centers for Disease Control and Prevention or the Agency for Toxic Substances and Disease Registry.

References:
Introduction: Human biomonitoring data, reflecting real-life exposure to chemicals, commonly shows that individuals are exposed to more than one PFAS simultaneously.

Materials and Methods: The Real-life mixture project in PARC (https://www.eu-parc.eu/) aims to develop a strategy to perform mixture risk assessment by applying human biomonitoring data, combining knowledge developed for mixtures in regulatory risk assessment, toxicology, and epidemiology. Statistical analysis (such as mixture identification), kinetic modelling, link with biomarkers of effect, and risk assessment will be performed in a harmonized way using the Monte Carlo Risk Assessment (MCRA) toolbox (https://mcra.rivm.nl/).

Results: We present a first outline of the strategy for PFAS mixture risk assessment specifically for human biomonitoring data. Relative potency factors (RPFs) for immunotoxicity derived at internal (blood) level will be combined with the EFSA tolerable weekly intake (TWI) expressed at blood level to interpret the risk of immunosuppression from exposure to real-life mixtures of PFAS.

Discussion and Conclusion: The traditional approach towards chemical risk assessment is performed on a substance-by-substance basis, consequently underestimating the risk from exposure to PFAS mixtures with similar adverse health effects. The MCRA software provides tools to address the health risk of aggregate and cumulative exposure to PFAS.
**WED-AM-B3  Perfluorinated substances and hormone receptor-positive breast cancer**

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**Introduction:** Perfluorinated substances (PFAS) are persisting chemicals with endocrine disruptive properties. There is limited and conflicting evidence on breast cancer risk, mostly coming from studies at background exposure levels [1, 2, 3]. Previous studies in highly exposed cohorts have not shown an increased breast cancer risk [1, 3]. No studies have explored the association between hormone receptor positive breast cancer and high PFAS exposure.

**Materials and Methods:** In 2013, high levels of PFAS (sum of PFAS > 10,000 ng/L), dominated by perfluorooctanesulfonic acid (PFOS) and perfluorohexanesulfonic acid (PFHxS), were found in the drinking water from one of the two waterworks in Ronneby, Sweden. Females (n = 29,856) who had ever resided in the municipality between 1985 and 2013 formed a cohort [3]. Individual exposure was assessed based on municipality waterworks distribution data linked to annual residential addresses. Breast cancer diagnoses were retrieved from the Swedish National Patient Register (1985-2016), and the Swedish Cancer Registry (1985-2016), while the Prescription database was available only from 2006 and on. Information on highest achieved educational levels was used as an indicator of socioeconomic position. No data on individual risk factors were available. Cox proportional hazards models were used in the analysis.

**Results:** There were 313 cases of breast cancer among women ≤85 years between 2006 and 2016. Of these, 224 cases (72%) were considered hormone receptor positive based on first prescription of adjuvant anti-hormonal drugs (antiestrogens (40%) or aromatase inhibitor (60%)). Among these 224 cases, the mean age at diagnosis was 62 years. The hazard ratio (HR) for this group was 0.84; 95% confidence interval (CI) 0.61, 1.14, p=0.26. Respectively, 28% of the cases were classified as non-hormone receptor positive breast cancer with mean age of 60 years. The HR for these cases were 1.35; 95% CI 0.87, 2.10, p=0.19.

**Discussion and Conclusion:** High PFAS exposure from drinking water in Ronneby was neither associated with an elevated risk of total breast cancer [3], nor with hormone receptor positive breast cancer or with non-hormone receptor positive breast cancer. Limitations of this registry study include the crude but individually modelled exposure, which however corresponds well to measured serum levels [4]. Adjustment for education partially addressed socioeconomic confounders. Together with previous research, our findings do not support an increased risk for breast cancer after environmental PFAS exposure.
**WED-AM-B5  Polychlorinated biphenyl biomonitoring in demolition workers**

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**Introduction:** Polychlorinated biphenyls (PCBs) are banned globally but are still present in buildings due to historical applications. At the Danish high-rise housing estate Brøndby Strand Parkerne, PCB indoor air measurements exceeded regulatory guideline levels, leading to the buildings’ demolition.¹ Our ongoing study conducts yearly biomonitoring among demolition workers to evaluate PCB exposure over the 3-year process. This includes demolition workers who are demolishing the five PCB-contaminated Brøndby Strand apartment buildings as well as additional sites with unknown PCB status. The central hypothesis evaluated here is that demolition workers may have increased and accumulating exposure to PCBs compared to a matched population of office workers from the demolition industry.

**Materials and Methods:** Blood samples were collected among demolition workers (both at Brøndby Strand Parkerne and from additional sites with unknown PCB status) and from a matched reference group of office workers from the demolition industry (n=62 participants, total). Additional information on general work tasks, potential previous exposure, and dietary habits were also collected from all participants. Blood samples from the first two years were extracted and analysed via GC-MS/MS for all 209 PCB congeners.

**Results:** Approximately 50 PCB congeners were detected in over 50% of the serum samples, from both demolition workers and office workers. At baseline sampling, the two groups had similar concentrations of total serum PCBs (geomean=98 ng/g lipid). Time trends from baseline through the first sampling year require further detailed investigation, particularly in examining specific homologue groups; however, preliminary analyses suggest an increasing trend in all demolition workers, which was not observed among the office workers.

**Discussion and Conclusion:** Recognition of the potential exposure to PCBs during demolition work and handling of demolition waste has been documented; however, there has been limited longitudinal study of bioaccumulation of PCBs from demolition work specifically. Serum PCB concentrations of all workers were in general lower than the residents who had previously lived in these apartment buildings as well as measurements of total PCBs in office workers of contaminated buildings.²,³ Nonetheless, any additional PCB accumulation among demolition workers compared to office workers during the project would prompt additional investigation into potential preventative exposure measures. Destruction and remediation processes for PCBs are critical to meeting Stockholm Convention guidelines. Our study provides preliminary insights on the potential PCB exposure burden among demolition workers of contaminated buildings, and also demolition workers more generally.

**Acknowledgments:** Sample collection was funded by the Danish Working Environment Authority (AT), while sample and data analyses were funded by the Working Environment Research Fund (AMFF, grant #20205100184). The authors further acknowledge Ulla Tegner for her contributions to the serum collection and preparation.

**References:**


1. Introduction:
Polychlorinated naphthalenes (PCN) were produced during much of the last century until the 1980s. They were used in multiple applications such as adhesives, dielectrics, flame (fire) retardant, fuel additives, fungicides, impregnating agents, insecticides, lubricants, plasticizers, solvents, stabilizers, wood preservatives, etc. The combined total global production volumes of technological formulations were estimated to range from 150,000 to 400,000 tons (Falandysz, 1998; Falandysz and Fernandes, 2020). In addition to this industrial production, PCN are also formed and released unintentionally during combustion processes and as by-products during the manufacture of other large-volume industrial chemicals such as polychlorinated biphenyls (PCB). The production, properties, applications, environmental distribution, contamination of food, occurrence in human tissues and other relevant background information on PCN has been extensively described in earlier reviews (Jakobsson and Asplund, 2000; Falandysz, 2003; Fernandes et al., 2017).

In 2015, PCN were listed to Annexes A (Elimination) and C (Unintentional release) under the Stockholm Convention. All listed POPs, including PCN are subject to Article 16 of this Convention, which requires that they should be monitored in order to evaluate the effectiveness of the remedial actions applied. The analysis of these POPs in human milk as one of the core monitoring matrices has been recommended within the framework of the Global Monitoring Plan on POPs (GMP) (UNEP, 2019).

Exposure to PCN reportedly provokes a number of toxic responses ranging from hepatotoxicity, neurotoxicity and immune response suppression along with endocrine disruption. Exposure to some PCN congeners results in toxicological responses that are similar to 2,3,7,8-TCDD (dioxin-like toxicity) (Engwall et al., 1994; Villeneuve et al 2000; Suzuki et al., 2020; Fernandes et al., 2022). The most studied of these PCN congeners have the ability to bind with varying degrees of potency to the aryl hydrocarbon receptor (AhR) in line with their PCDD/PCDF analogues, the most potent of which include congeners such as 2,3,7,8-Tetrachlorodibenzo-p-dioxin (2,3,7,8-Tetrachlorodibenzo-p-dioxin), 1,2,3,7,8-PentaCDD, etc. The preliminary stage of the biochemical mechanism that initiates many of the potent long-term toxic effects of these compounds on vertebrate species is through activation of the AhR. Therefore, the inclusion of PCN in the Toxicity Equivalency Factor (TEF) concept for dioxin-like compounds has been suggested (van den Berg et al., 2006).

Between 2000 and 2019, the World Health Organization (WHO) and the United Nations Environment Programme (UNEP) have coordinated five global exposure studies on POPs in human milk. In a series of publications, the concept, analysis, results and discussion of these studies for 32 POPs are described in a compendium “Persistent organic pollutants in human milk” (Malisch, Fürst and Šebková, 2023), including a chapter on polychlorinated naphthalenes. For overall conclusions and key messages of these studies, see Malisch et al. 2023.

The data are publicly available in the Data Warehouse of the Stockholm Convention Global Monitoring Plan (GMP DWH) (GMP DWH, 2020).

This article discusses the PCN results for 40 pooled human milk samples from 39 countries collected between 2016 and 2019.
milk studies 2000–2019 are described in the compendium. A set of 26 PCN congeners (Table 1) were selected on the basis of the toxicological characteristics, reported levels of occurrence, congener patterns and the availability of analytical standards during the period of method development and validation.

<table>
<thead>
<tr>
<th>Congener number</th>
<th>Chemical structure</th>
<th>Congener number</th>
<th>Chemical structure</th>
</tr>
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<tbody>
<tr>
<td>PCN 27</td>
<td>1,2,3,4-TetraCN</td>
<td>PCN 63</td>
<td>1,2,3,4,5,6-HexaCN</td>
</tr>
<tr>
<td>PCN 28/36</td>
<td>1,2,3,5-TetraCN/1,2,5,6-TetraCN</td>
<td>PCN 64/68</td>
<td>1,2,3,4,5,7-HexaCN/1,2,3,5,6,8-HexaCN</td>
</tr>
<tr>
<td>PCN 31</td>
<td>1,2,3,8-TetraCN</td>
<td>PCN 65</td>
<td>1,2,3,4,5,8-HexaCN</td>
</tr>
<tr>
<td>PCN 42</td>
<td>1,3,5,7-TetraCN</td>
<td>PCN 66/67</td>
<td>1,2,3,4,6,7-HexaCN/1,2,3,5,6,7-HexaCN</td>
</tr>
<tr>
<td>PCN 46</td>
<td>1,4,5,8-TetraCN</td>
<td>PCN 69</td>
<td>1,2,3,5,7,8-HexaCN</td>
</tr>
<tr>
<td>PCN 48</td>
<td>2,3,6,7-TetraCN</td>
<td>PCN 70</td>
<td>1,2,3,6,7,8-HexaCN</td>
</tr>
<tr>
<td>PCN 49</td>
<td>1,2,3,4,5-PentaCN</td>
<td>PCN 71/72</td>
<td>1,2,4,5,6,8-HexaCN/1,2,4,5,7,8-HexaCN</td>
</tr>
<tr>
<td>PCN 50</td>
<td>1,2,3,4,6-PentaCN</td>
<td>PCN 73</td>
<td>1,2,3,4,5,6,7-HeptaCN</td>
</tr>
<tr>
<td>PCN 52/60</td>
<td>1,2,3,5,7-PentaCN/1,2,4,6,7-PentaCN</td>
<td>PCN 74</td>
<td>1,2,3,4,5,6,8-HeptaCN</td>
</tr>
<tr>
<td>PCN 53</td>
<td>1,2,3,5,8-PentaCN</td>
<td>PCN 75</td>
<td>OctaCN</td>
</tr>
<tr>
<td>PCN 59</td>
<td>1,2,4,5,8-PentaCN</td>
<td></td>
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</tr>
</tbody>
</table>

Table 1: PCN congeners covered by the applied analytical method. Congeners that based on their chlorine substitution pattern are expected to produce dioxin-like effects are indicated in bold; congener pairs that were not chromatographically separated during measurement are marked as *).

3. Results, Discussion, and Conclusions:
3.1 Sum of measured PCN congeners (Σ 26 PCN)
The median concentration of Σ26 PCN in 40 pooled human milk samples from 39 countries was 55 pg/g lipid (range 27 pg/g to 170 pg/g). The concentrations in samples from European countries were considerably higher than those found in the other regions included in this study (Africa, Asia-Pacific and Latin America and Caribbean). The median concentration for Europe, at 152 pg/g, was three-fold higher than found in the other regions. This higher PCN contamination in European human milk is reflected in the minimum concentration which at 86 pg/g is very close to the mean maximum level (93 pg/g) for the other three regions. This finding is not unexpected as the European region saw high levels of manufacture and use of PCN during the last century, as was reported earlier for North America. In the non-European countries included in this study (which do not include the North American region), the concentrations of Σ26 PCN were relatively low, ranging from 27 pg/g to 66 pg/g, apart from the Solomon Islands and Jamaica.

3.2 PCN patterns
The most abundant congeners were the congener pairs PCN 52/60 and PCN 66/67 (currently inseparable by conventional chromatography) and to a lesser extent PCN 28/36, PCN 42, PCN 46, PCN 48, PCN 59 and PCN 69.

3.3 Dioxin-like properties – determination of PCN-TEQ
Among other adverse biological effects, a critical response of many PCN congeners is dioxin-like toxicity. So, in addition to reporting concentrations of individual congeners, the toxic equivalents (TEQs) were also calculated in these samples, using two sets of relative effect potency (REP) values: a set that has been used in a number of human exposure studies (e.g. Falandysz et al., 2019; Falandysz and Fernandes, 2020; Fernandes et al., 2011, 2022; Zacs et al., 2021) and another set reported by Falandysz et al, (2014). These sets were derived from a number of in vitro and in silico studies and the single in vivo study deriving REPs for PCN 66 and 67 (Hooth et al., 2012).

As these REP for PCN have been derived mostly from in vitro studies, applicability to in vivo conditions, especially in those situations when prolonged exposure takes places, e.g. a breastfed infant, may introduce uncertainties to the interpretation of the effects of exposure. The only available in vivo study covered biological as well as toxicological endpoints for PCN-66 and PCN-67 after a two week oral exposure (Hooth et al., 2012).

Both sets of REPs, those used in human exposure studies and those proposed by Falandysz et al. (2014) were used for the
TEQ calculations of the WHO/UNEP human milk samples. For PCN 66, the REPs of 0.004 (human exposure studies) and 0.002 (Falandysz et al., 2014) are in good agreement with the in vivo study (range 0.0015-0.0072, Hooth et al., 2012). For PCN 67, the REP values used are also 0.004 and 0.002, but they are higher than the range reported (0.0003-0.0007) from the in vivo study. As PCNs 66 and 67 are chromatographically inseparable under routine conditions, the human exposure studies follow the precautionary principle and used the higher of the two REP values for estimating TEQ. Additionally, the in vivo REP values are derived from a single study, as compared to the in vitro REPs that consider data from a number of studies.

The use of the in vitro REP for PCN 67 may lead to some overestimation of the calculated PCN-TEQ in human milk depending on the extent to which it contributes to the summed PCN-TEQ. The individual contribution of PCN 66 and PCN 67 to the TEQ in human milk could be refined by considering a REP value for PCN 67 that was closer to the range from the in vivo study if the individual concentrations were known. However, a chromatographic separation of PCN 66 and 67 is only possible by using a special GC column with considerable limitations under routine conditions, so in practice, it is only possible to determine the sum of the two congeners. PCNs 66 and 67 were separated in commercial PCN Halowax products using the specialized conditions (Helm et al., 1999) and the mass percent contribution of PCN 66 to the sum (of PCNs 66 plus 67) was 55 to 60%. For a congener-specific assessment, this ratio was used to estimate the individual concentrations of PCNs 66 and 67.

The median PCN-TEQ concentration in human milk was 0.07 pg PCN-TEQ/g lipid (range 0.03 pg/g to 0.23 pg/g), when calculated using the human biomonitoring study REPs, and 0.03 pg PCN-TEQ/g lipid (range 0.01 pg/g to 0.10 pg/g), when calculated with other suggested REPs.

In conclusion, resolution of the value of the REPs used is more important than the separate determination of PCN 66 and 67. Additional in vivo data for all contributing congeners would reduce the uncertainty of the current REPs and hence the TEQ. PCN-TEQ based on the two sets of REPs differ by a factor of 2.2 (as median of the factors for the individual country results obtained by the standard method), whereas the congener-specific determination is expected to result in differences of approximately 30% to the standard method. These uncertainties can only be reduced by (i) further studies on PCN REPs, preferably under in vivo conditions which would allow consensus values to be established (ii) analytical research and method development allowing the determination of PCN 66 and 67 in human milk samples separately.

On average, the contribution of PCN-TEQ to the cumulative TEQ (including the overall sum of toxic equivalents of PCDD, PCDF and dioxin-like PCB [WHO2005-TEQ]) is between 1% and 2%, with a wider range of up to 5% for the 39 countries of this study. This is about an order of magnitude lower than the contribution of dioxin-like PCB to the cumulative TEQ (median 26%). European countries also showed considerably higher levels of PCN-TEQ compared to the other regions. PCN-TEQ calculated with REPs used in human biomonitoring studies add on average about 2% to the cumulative TEQ of dioxin-like contaminants in Africa, the Asia-Pacific region and Latin American and Caribbean countries and about 4% in European countries. The corresponding contribution of PCN-TEQ calculated using the other set would be 1% in non-European countries and 2% in European countries.

4. Acknowledgments:

We acknowledge the contribution and support from WHO and UNEP for the exposure studies on human milk coordinated in the period 2000-2019, the national coordinators, assisted by the respective health, laboratory, and administrative staff, furthermore the team at CVUA Freiburg for the performance of the analyses of the POPs – and last, but not least, all mothers providing human milk.

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Introduction: The harbour seal (Phoca vitulina) and the grey seal (Halichoerus grypus) in the North Sea and Northeast Atlantic are especially vulnerable to persistent organic pollutants (POPs) because they are long-lived top predators and contain considerable levels of fat which accumulate lipophilic pollutants. The potential impacts of these substances on vulnerable species such as the harbor seal and grey seal in this region highlight the importance of continuing to monitor, track, and mitigate their effects. The present study aimed to compare the levels of POPs (PCBs, PBDEs, HCHs, HCB, DDTs, CHLs, and PBDEs) in tissues of the grey seal and the harbour seal from Scotland. Furthermore, biotic factors such as region, potential diet, seal body mass, body size, and sex were also examined for their influence on POP contamination.

Materials and Methods: In 2017, blubber was sampled from 34 free-ranging adult harbour seals (17 males and 17 females, Orkney, Northeast Atlantic) and 34 free-ranging adult female grey seals (Isle of May, North Sea) in 2017 (permit 60/3303). Twenty-eight PCB congeners (IUPAC numbers: CB 28, CB 52, CB 47, CB 49, CB 66, CB 74, CB 99, CB101, CB 105, CB 110, CB 118, CB 128, CB 138, CB 146, CB 149, CB 153, CB 156, CB 170, CB 177, CB180, CB 183, CB 187, CB 196/203, CB 194, CB 199, CB 206, CB 209), 3 DDTs (p,p′-DDD, p,p′-DDE, and p,p′-DDT), chlordane compounds (CHLs) (oxychlordane (OxC), trans-nonachlor (TN), cis-nonachlor (CN), cis-chlordane (CC), and trans-chlordane (TC)), 3 hexachlorocyclohexane (HCH) isomers (∼-HCH, ∼-HCH and ∼-HCH), hexachlorobenzene (HCB), as well as 7 PBDEs (IUPAC numbers: BDE 28, 47, 99, 100, 153, 154, and 183), were targeted in the blubber 1. The six indicator PCBs were CB-28, PCB-52, PCB-101, PCB-138, PCB-153 and PCB-180 which are further referred to as ∑6PCBs. Additionally, two MeO-PBDEs (2′-MeO-BDE 68 and 6-MeO-BDE 47) were also determined. ∼13C, ∼15N and ∼34S values were analyzed in hair and blood cells 2.

Results: Analysis of blubber samples from both the Orkney Islands and the Isle of May indicated variable concentrations of POPs, with PCBs being highest, followed by DDTs, CHLs, PBDEs, HCBs, MeO-PBDEs, and HCH. Male harbour seals living on Orkney had significantly higher concentrations of PCBs than female counterparts (Mean ∑PCB: 2.45 vs 1.29 µg.g-1 lipid), while females on Orkney had lower ∑6PCB concentrations than females on Isle of May (mean: 0.85 vs 1.26 µg.g-1 lipid). Additionally, body size in harbour seals was found to be correlated with both ∑6 PCB and ∑CHL concentrations as a possible proxy for age.

Discussion and Conclusion: Our results suggest that there is a higher level of POP exposure among the seals from Isle of May as compared to those from Orkney due to anthropogenic activities which has been supported by earlier data3,4. In addition, our findings point towards lower POP exposure levels found among Scotland’s seals than those sampled in the southern part of the North Sea 3,4.

Acknowledgments: The authors would also like to thank Simon Moss and all involved in IoM fieldwork (SMRU, St Andrews) for their assistance with sample collection of seals, respectively. All capture and handling procedures were performed under the UK Home Office licence (permit: # 60/3303) in Scotland. Funding was supported by F.R.S.-FNRS (Belgium) and Natural Environment Research Council UK core grant to SMRU.

References:
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1. Introduction:
Persistent Organic Pollutants (POPs) have attracted global attention in recent decades owing to their bioaccumulation properties, high toxicity and ubiquitous distribution in the environment and biota [1]. Among POPs, brominated flame retardants (BFRs) and perfluoroalkyl substances (PFASs) are emerging contaminants listed in the Stockholm Convention [2-8]. BFRs are used as additives in a wide variety of commercial and industrial products to inhibit or delay the spread of fire. The oldest and most widely used BFRs are polybromodiphenyl ethers (PBDEs) and hexabromocyclododecane (HBCDs) [9]. PBDEs are a family of 209 possible congeners produced in 3 major technical mixtures, with varying bromination degrees (penta-, octa- and deca-BDE) [10], while HBCDs technical products contain 3 main isomers: g (75-89%), a (10-13%) and b (1-12%) [11-12]. Concerns have been raised regarding BFRs potential dioxin-like toxicological properties and their endocrine-disrupting effects. BDEs and HBCDs were therefore both incorporated into the Stockholm Convention elimination list [2, 4, 6, 8]. As a result of these bans, the use of other flame retardants, classified as “emerging” BFRs (eBFRs), increased. Important representatives of this group are Bis(2,4,6-tribromophenoxy)ethane (BTBPE), decabromodiphenyl ether (DBDPE), Hexabromobenzene (HBBz), pentabromobenzene (PBBz), pentabromoethylen (PBE), pentabromotoluene (PBT), hexabromocyclopentadienyl dibromocyclooctane (HCBDCO), 2-ethylhexyl 2,3,4,5-tetrabromobenzoate (EHTBB) and 2,3,5,6-tetrabromo-p-xylene (pTBX). Due to their increasing use, traces of these compounds have been detected in environmental matrices. Studies have proven that many eBFRs have properties similar to POPs and raise potential human health concerns [13].

PFASs are highly fluorinated aliphatic compounds synthesized on a large scale and widely used in many chemical processes and industrial applications [15]. They are widely detected in the environment due to their resistance to degradation and widespread use; they resulted to be toxic and bio-accumulative in biota and humans [15]. PFOS (perfluorooctanesulfonic acid), PFOA (perfluorooctanoic acid) and PFHxS (perfluorohexane sulfonic acid), were listed or recommended for listing in the Stockholm Convention [3,5,7].

Wild animals can be exposed to POPs and the levels in their tissues may represent a suitable indicator of environmental pollution. Moreover, game animals may also be a source of exposure for certain selected populations, such as hunters [16]. To the best of our knowledge, to date studies in central Italy have been performed only on wild boar [17]. This study reports preliminary results on PFASs, PBDEs, HBCDs and eBFRs measured in muscle and liver of three different wild species, caught in the Central Apennine Mountains (Italy).

2. Materials and Methods:
2.1 Sampling
A total of fourteen wild animals were analyzed, including ten specimen of roe deer (Capreolus capreolus), three of red fox (Vulpes vulpes) and one European hedgehog (Erinaceus europeus). The individual specimens were collected in central Italy’s Apennine Mountains. The species were selected according to their different eating habits: herbivores (roe deer), carnivorous (fox) and omnivorous (hedgehog). Muscle and liver tissues were collected, homogenized, frozen (-20°C) and stored in laboratory until analysis. Gender and age of each animal were recorded.

2.2 Brominated flame retardants analytical method
Nine BDE congeners (28, 47, 49, 99, 100, 153, 154, 183, 209), 3 HBCD isomers (a, b and g) and 9 eBFRs (pTBX, PBBz, PBT, PBE, HBBz, EHTBB, HCBDCO, BTBPE and DBDPE) were analyzed in isotopic dilution with a single preparation followed by a dual detection in GC-MS/MS (PBDEs and eBFRs) and LC-MS/MS (HBCDs). The analytical method for PBDEs and HBCDs analysis was already thoroughly described [18]. The same condition were applied with good results to the analysis of the eBFRs. Muscle and liver samples were subjected to QuEChERS-like extraction and to a double clean-up on acidified Extrelut NT-3/SPE Si 1 g/6 mL tandem columns assembly followed by gel permeation chromatography. Each purified extract was divided into two fractions and reduced to dryness. PBDEs/eBFRs fraction was analyzed in GC-MS/MS on a 7890A GC – 7000B triple-quadrupole mass analyser (Agilent Technologies, Palo Alto, California, U.S) [19]. The HBCDs fraction was injected in a LC-MS/MS ACQUITY I-Class Ultra Performance Liquid Chromatography/ Xevo
WED-AM-C2  Brominated flame retardants (PBDEs, HBCDs and eBFRs) and perfluoroalkyl substances (PFASs) in wild animals from central Italy. Are they contamination sentinels?

TQ-S micro IVD system (Waters, Milford, Massachusetts, U.S.) [18].

2.3 Perfluoroalkyl substances analytical method

11 perfluoroalkyl carboxylic acids (PFCAs: PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUdA, PFDoA, PFTrDA, PFTeDA) and 8 perfluoroalkane sulfonic acids (PFSAs: L-PFBS, L-PFPeS, L-PFHxS, L-PFHxS, L-PFOS, L-PFNS, L-PFDS and L-PFDoS) were analysed in isotopic dilution as previously described [16-19]. Branched PFOS (br-PFOS) isomers and PFOSA were also quantified. Muscle and liver samples were extracted twice in ultrasound bath with acetonitrile and purified on a Strata X-AW SPE cartridge (200 mg/6 mL), eluting with 2% ammonium hydroxide in methanol and subjected to dispersive SPE with Envicarb® and acetic acid. Prior to elution, the SPE column was washed with methanol, and this fraction was separately collected for PFOSA analysis. The instrumental analysis was performed on an ACQUITY I-Class-LC/Xevo TQ-S micro IVD system in ESI negative mode [17-20].

2.4 Quality Assurance/Quality Control

Background contamination was carefully monitored at any stage of the analytical process and a strict quality control was implemented in each batch [17-19]. Limits of quantification (LOQs) were estimated on spiked samples: 0.005 mg/kg for PBDEs (209: 0.10 mg/kg) and 0.010 mg/kg for HBCDs, eBFRs (BTBPE: 0.020 mg/kg; DBDPE: 0.10 mg/kg) and PFASs (PFBA: 0.20 mg/kg; PFOSA: 0.050 mg/kg). Regular participation to inter-calibration exercises organized by the EURL ensured external quality assurance.

3. Results:

PFAS levels and the contamination pattern in both muscle and liver were reported in Figure 1 (for red fox and roe deer the median value was reported). In muscle, PFASs were not quantified in roe deer, while in red fox SPFASs lower bound (l.b.) ranged between 0.48-1.3 mg/kg and 0.91 mg/kg were measured in the only hedgehog analysed (Fig.1). As regard BFRs, traces of a-HBCD were measured only in hedgehog (0.018 mg/kg). PBDEs and eBFRs were all below LOQs in all the samples analyzed.

In liver, significantly higher SPFASs level were measured respect to muscle, in all the considered species. Concentrations between 0.072-1.9 mg/kg and 5.2-17 mg/kg were found in roe deer and red fox respectively. The only hedgehogs showed a level of 7.8 mg/kg. SPBDEs l.b. was negligible in roe deer, while in fox and hedgehog was 1.1 (median) and 0.46 mg/kg respectively. In both these last species 0.019 and 0.18 mg/kg of a-HBCD were measured as median. Only in roe deer livers eBFRs were found above the LOQ: one sample showed pTBX, PBT, PBEB and EHTBB at 0.039, 0.084, 0.092, 0.047 mg/kg respectively; EHTBB (0.030 mg/kg) was measured in another sample and DBDPE in two other specimen at 0.34 and 0.40 mg/kg. As regard red foxes and hedgehog PBDEs were measured at low levels with the following contamination pattern: BDE-209>-153>-47 (Fig.2).

![Figure 1: PFASs concentration and contamination pattern in muscle and liver (red fox and roe deer median values)
WED-AM-C2  Brominated flame retardants (PBDEs, HBCDs and eBFRs) and perfluoroalkyl substances (PFASs) in wild animals from central Italy. Are they contamination sentinels?

4. Discussion:
As regard PFASs, in all the three species PFOS was the most prevalent compound in both tissues, followed by PFTrDA (C13) and the other long chain PFCAs (C8-C14). Short-chain PFAS and PFOSA were not quantified, according to their low bioaccumulation potential [15]. The contamination pattern in muscle and liver was comparable, unlike what previously reported for wild boar, where PFTrDA was the dominant compound in muscle [17].

Significantly higher levels were measured in liver than in muscle, in line with the high PFASs affinity for liver fatty acid-binding proteins [15]. PFASs concentration in fox (carnivorous) and hedgehog (omnivorous) livers are significantly higher than in roe deer (herbivorous) (Fig.3). This difference could be the result of different feeding habits. A preferential accumulation of L-PFOS rather than br-PFOS was found in muscle and liver of fox and hedgehogs, where the br-PFOS/PFOS ratio was estimated to be 4-15%, consistently with what already found in wild boar (br-PFOS/PFOS = 6-10%) [17]. Completely different was in roe deer’s liver pattern where the br-PFOS concentration was comparable or higher respect to the linear, and the ratio br-PFOS/PFOS was included between 91-146%.

No correlation between PFASs concentration and animal age or gender was observed, but this may be because of the few preliminary data collected.

BFRs levels were generally lower than PFAS. Differently from what previously reported for wild boar, BFRs levels in muscle were negligible: only traces of α-HBCD were measured in the hedgehog [17]. This is quite surprising, because BFRs, like most persistent organic contaminants, are lipophilic and tend to accumulate primarily in muscle and adipose tissue rather than in the liver [10-13]. Very low levels of PBDEs and HBCDs were quantified in the herbivorous roe deer’s liver, while higher concentrations were measured in fox’s and hedgehogs’s. α-HBCD was the dominant isomer, as already reported [17-18]. Interestingly, in livers of both the above mentioned species, the most abundant PBDE congeners were BDE-209 and -153, and not the 47, which is usually the most abundant in muscle [18-19]. Unexpectedly, eBFRs were above LOQs only in roe deer livers. Specific reasons were not identified, considering that no data are published to date on eBFRs in wild terrestrial animal.

Extremely limited information is available on these wild species from Europe. The here reported PFAS levels are lower than that measured in roe deer’s liver from Germany, where PFOS and PFOA ranged between 1.3-67.5 mg/kg and <0.2-3.2 mg/kg respectively [21-22]. Higher than the results here reported were also the level reported in German fox liver (PFOS: 3.2-320 mg/kg; PFOA<LOQ-2.0 mg/kg). Comparable were the PFAS and BFR (PBDE and HBCD) concentrations in roe deer muscle sampled in an Italian subalpine area [16]. In the best of our knowledge, no data were yet published on eBFRs in wild fauna in Europe.
5. Conclusions:
Fourteen samples of roe deer, red fox and hedgehogs muscles and livers were analyzed for PFASs, PBDEs, HBCDs and eBFRs, in order to study wild life contamination levels in Central Italy and evaluate the possible use of game species as environmental contamination indicator. This is the first report which includes also eBFRs levels in terrestrial wild life.

PFAS were generally higher than BFRs, and liver was more contaminated than muscle, confirming that liver, a protein rich tissue, is the target organ for PFASs accumulation. Among the species analysed the herbivorous roe deer shows lower contaminants levels, with the exception of eBFR in one liver sample. The obtain results seems interesting encouraging further investigation.

6. Acknowledgments:
The authors gratefully acknowledge financial support from the Italian Health Ministry: IZS UM 02/21 RC - WildSENTINEL-2021

7. References:
5. UNEP, 2013. Perfluorohexane sulfonic acid (PFHxS), its salts and PFHxS-related compounds. SC-10/13.
WED-AM-C2  Brominated flame retardants (PBDEs, HBCDs and eBFRs) and perfluoroalkyl substances (PFASs) in wild animals from central Italy. Are they contamination sentinels?

Introduction: The Convention for the Protection of the Marine Environment of the North-East Atlantic ("OSPAR Convention") established several environmental indicators to monitor persistent chemicals (PCs, e.g. PCBs and mercury) in European waters. Due to their long lifespan, high trophic position and large blubber reservoirs, marine mammals may accumulate extremely high concentrations of PCs, often surpassing established toxicity thresholds. At the same time, they can help to assess the pollution state of the entire food web in an integrated manner. This work aimed at collecting and harmonizing the existing data on PCs' pollution in marine mammals across all OSPAR-managed regions in order to develop the framework for a new pollution indicator for monitoring the environmental status of the Northeast Atlantic.

Materials and Methods: Data covering all five OSPAR regions (I: Arctic waters, II: Greater North Sea, III: Celtic Sea, IV: Bay of Biscay and Iberian Coast, V: Wider Atlantic) was provided by ten OSPAR contracting parties (B, DE, DK, ES, FR, IE, NL, PT, SE, and UK) for 30 species of cetaceans and pinnipeds. Of all PCs analyzed (>200), PCBs had the widest data coverage and were selected as sentinel PCs to develop the conceptual framework of the indicator. The sum of total PCBs concentrations (ΣPCBtot) was explored, with the number of analyzed PCB congeners varying from 6 to 48. We performed a preliminary qualitative analysis of marine mammal’s pollution status, structuring the indicator’s framework in three parts: spatial, temporal and status assessment.

Results: The highest availability of PCBs data was found for the 2009-2016 time-frame and for regions II, III and IV3. Scarce data was available for Region I and V. Small toothed cetaceans presented the highest range of the ΣPCBtot (0.20 – 820 mg/kg lipid weight, lw), followed by pinnipeds (0.01 – 226 mg/kg lw), deep-diving cetaceans (0.01 – 72 mg/kg lw), and baleen whales (0.04 – 21 mg/kg lw), all ages and sexes lumped for each group. The ΣPCBtot surpassed the established toxicity range for reproductive impairment (9 – 41 mg/kg lw) in most small toothed cetaceans, in some deep-divers and in a few pinnipeds. Baleen whales did not surpass known toxicity thresholds.

Discussion and Conclusion: Although data are scattered and not all species represented, the ΣPCBtot seemed to be influenced by ecological traits (e.g. diet and habitat use) rather than region or year. This preliminary assessment underlines the need for future efforts towards data harmonization at regional European level. Several parties reported that many samples are available in their tissue banks that have not been analyzed due to budget limitations. This prompts the prioritization of funding projects aiming to fill gaps at temporal and spatial scale. The next steps will include the addition of legacy and emerging PCs into the spatial and tissue banks that have not been analyzed due to budget limitations. This prompts the prioritization of funding projects aiming to fill gaps at temporal and spatial scale. The next steps will include the addition of legacy and emerging PCs into the spatial and status assessment.

Acknowledgments: The authors thank the German Federal Agency for Nature Conservation (BfN) who funded the project and OSPAR for supporting and supervising the development of the indicator through the Marine Mammals Expert Group, Hazardous Substances and Eutrophication Committee, MIME, BDC and ICG-COBAM.

References:
1. Introduction:
Atmospheric deposition of substances emitted to the atmosphere can accumulate in soils and biomass such as mosses. Compared to deposition measurement networks with technical sampling devices, measurement networks with mosses provide a spatially much higher resolution. Mosses (Bryophyta, nonvascular plants) lack any roots. This is the prerequisite to infer on a compound’s accumulation in moss from atmospheric sources. Mosses absorb dry, wet or occultly deposited pollutants directly via their surface and accumulate them. Substance-resistant moss species such as Pleurozium schreberi (BRID.) MITT., Hypnum cupressiforme HEDW. s.str. and Pseudoscleropodium purum (HEDW.) M.FLEISCH (synonym Scleropodium purum HEDW. LIMPR.) have widespread occurrence and proved to be good accumulation indicators for substances like metals, nitrogen, persistent organic pollutants and microplastics1. Bioindication with mosses has financial advantages over technical methods for quantifying atmospheric deposition and is therefore well suited for detecting large-scale trends in the bioaccumulation of atmospheric deposition in spatially dense monitoring networks. Consequently, in 1987 the International Cooperative Programme on Effects of Air Pollution on Natural Vegetation and Crops (ICP Vegetation) was established to investigate the scientific basis for quantifying the damage to plants associated to the deposition of air pollutants. In 2001, ICP Vegetation took on responsibility from the Nordic Council of Ministers for coordinating the Europe-wide determination of heavy metals in mosses, which has been carried out every five years since 1990 and has included a maximum of about 7300 moss sampling sites, compared to about 60 deposition monitoring sites of the European Monitoring and Assessment Programme throughout Europe2. Since 2005, nitrogen concentrations in mosses have also been recorded. Persistent organic pollutants were included in 2010 (2015 in Germany), and pilot studies on microplastic followed in 2015 (2020 in Germany) 1,3.

The ICP Vegetation is part of the activities of the Working Group on Impacts under the Convention on Long-Range Transboundary Air Pollution, which covers the UNECE (United Nations Economic Commission for Europe) region in Europe and North America. The protocols of the Convention commit countries to reducing pollutant emissions by certain target years. The results of the ICPs and their annual task force meetings are used to monitor the reduction of air pollutants and their impact on health and the environment. This requires reliable data on spatial and temporal trends of substance accumulation in ecosystems. The present study was carried out within the German Moss Survey from the beginning of October 2020 to the end of 2023. Heavy metals, nitrogen, POPs and microplastics were investigated at selected locations. Here, we are presenting results for a variety of persistent organic pollutants (POP), flame retardants in particular, map spatial patterns of their accumulation and compare them temporally with respective data collected in the Moss Surveys 2015.

2. Materials and Methods:
Briefly, sampling was performed at 21 sites according to the recommendations of the ICP Vegetation Moss Survey Manual1. These included seven sites that had already been sampled in the 2015 moss monitoring. The analytical spectra was based on that of the moss monitoring in 2015 and included polycyclic aromatic hydrocarbons (PAH), polychlorinated dibenzodioxins and -furans (PCDD/F), polychlorinated biphenyls (PCB), brominated and chlorinated flame retardants (polybrominated biphenyls (PBB), polybrominated diphenyl ethers (PBDE), various other alternative or emerging halogenated flame retardants (HFR) as well as perfluorinated alkyl substances (PFAS). The methods for sample preparation and chemical analysis generally corresponded to those of the moss monitoring 20154 with slight modifications. Overall, about 120 compounds were determined in these surveys. Additionally and conducted as pilot phase in the 2020 survey, pesticides were investigated for the first time in a few selected samples applying a screening approach on about 650 compounds as well as a target-based method on 27 legacy organic chlorine pesticides.

3. Results:
The highest concentrations were observed for PAH. Concentrations for the sum of eight high molecular weight PAH were between 11.6 ng/g dry weight (dw) and 304.4 ng/g dw with an average of 70.2 ng/ g dw and a median of 47.6 ng/g dw. Among the halogenated POPs, flame retardants clearly stand out, in particular decabromodiphenylethane (DBDPE), BDE 209 and 2,3-dibromopropyl-2,4,6-tribromophenyl ether (DPTE), hexabromocyclododecane (HBCD) and dechloran plus (DP). Concentrations of DBDPE were between < limit of quantification (LOQ) and 7650 pg/g dw with a mean of 2085 pg/g dw and a median of 1635 pg/g dw. Concentrations of DPTE were between < LOQ and 374 pg/g dw with a mean of 163 pg/g dw and a
median of 209 pg/g dw. Concentrations of HBCD were between 23.4 pg/g dw and 1215 pg/g dw with an average of 254 pg/g dw and a median of 173 pg/g dw. Concentrations of DP were between 20.7 pg/g dw and 635 pg/g dw with a mean of 179 pg/g dw and a median of 134 pg/g dw. Dioxin-like PCBs and PFAS were rarely found above the LOQ. PBB and DIN-PCB were always below the LOQ. Of the approximately 650 individual compounds examined in the pesticide screening of five selected moss samples, only aldrin was observed above the LOQ in one sample. Azoxystrobin, boscalid, carbandazim, tebuconazole, terbuthylazine and terbuthylazine desethyl could be detected in some cases, but concentrations could not be quantified. The target-based investigation of 27 legacy organochlorine pesticides in three selected moss samples also provided only isolated findings.

4. Discussion:
Findings of the 2020 moss monitoring largely confirm the results of the 2015 moss monitoring. For the first time, it was possible to identify substance-specific temporal concentrations variations (Table 1). For components that were frequently found in both surveys, the concentrations mostly decreased from the 2015 moss monitoring to the 2020 moss monitoring. Exceptions were the flame retardants PBT (values increasing at almost all locations), HBBz and DPTE (values increasing at 3 or 2 out of 7 locations) and DBDPE as a potential DecaBDE substitute (values increasing at 3 out of 7 locations). DP, also characterized as a DecaBDE substitute, was observed at significantly lower values at all locations.

The increased number of sampling sites in the 2020 moss monitoring compared to the moss monitoring in 2015 also allowed for an initial location-related assessment. The locations classified as urban showed the highest average concentrations for most pollutants/pollutant classes. Locations close to the sea often had the lowest average values.

In the moss monitoring 2020, a description of the spatial distribution of organic contaminants in moss samples from Germany was possible. The spatial distribution of the substances often, but not exclusively, showed a concentration gradient with elevated values in densely populated and industrialized areas of Western Germany to lower concentrations in eastern areas and enhanced values in Central Germany (example see Figure 1).

Table 1: Inferential statistical comparison of median POP concentrations at locations of MM2015 and MM2020 (Wilcoxon test for paired sample).
WED-AM-C4  Spatio-Temporal Distribution of Organic Pollutants in Moss Samples from the 2022 German Moss Survey

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>n</th>
<th>Median 2015</th>
<th>Median 2020</th>
<th>2020-2015 [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decabromodiphenylethane (DBDPE)</td>
<td>pg/g dw</td>
<td>4</td>
<td>770.2</td>
<td>1400.0</td>
<td>82</td>
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<tr>
<td>syn-Dechlorane Plus (syn-DP)</td>
<td>pg/g dw</td>
<td>6</td>
<td>59.6</td>
<td>32.5</td>
<td>-46</td>
</tr>
<tr>
<td>anti-Dechlorane Plus (anti-DP)</td>
<td>pg/g dw</td>
<td>6</td>
<td>203.5</td>
<td>149.0</td>
<td>-27</td>
</tr>
<tr>
<td>HBCD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>alpha-HBCD</td>
<td>ng/g dw</td>
<td>7</td>
<td>0.2753</td>
<td>0.0616</td>
<td>-78 **</td>
</tr>
<tr>
<td>beta-HBCD</td>
<td>ng/g dw</td>
<td>5</td>
<td>0.0844</td>
<td>0.0184</td>
<td>-78</td>
</tr>
<tr>
<td>gamma-HBCD</td>
<td>ng/g dw</td>
<td>7</td>
<td>0.3047</td>
<td>0.0698</td>
<td>-77 **</td>
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<td>PAH</td>
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</tr>
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<td>Anthracene</td>
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<td>0.8</td>
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<td>Fluoranthene</td>
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<td>7</td>
<td>23.69</td>
<td>13.8</td>
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<tr>
<td>Pyrene</td>
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<td>7</td>
<td>22.08</td>
<td>12.4</td>
<td>-44</td>
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<tr>
<td>Benzo[a]anthracene</td>
<td>ng/g dw</td>
<td>6</td>
<td>5.45</td>
<td>5.64</td>
<td>3</td>
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<tr>
<td>Chrysene</td>
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<td>14.53</td>
<td>6.87</td>
<td>-53</td>
</tr>
<tr>
<td>Benzo(b+j)fluoranthene</td>
<td>ng/g dw</td>
<td>7</td>
<td>23.05</td>
<td>10.8</td>
<td>-53</td>
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<tr>
<td>Benzo(k)fluoranthene</td>
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<td>5.53</td>
<td>3.82</td>
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<tr>
<td>Benzo(a)pyrene</td>
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<td>6.43</td>
<td>6.61</td>
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<tr>
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<td>10.7</td>
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<td>Benzo(ghi)perylene</td>
<td>ng/g dw</td>
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<td>11.05</td>
<td>4.85</td>
<td>-56</td>
</tr>
</tbody>
</table>

n = sample size (paired samples). Values < LOQ were set to 0 for the characteristic value calculations; POPs with median values < LOQ were not considered for the inferential statistical comparison. *** = p ≤ 0.01 (very significant); ** = p ≤ 0.05 (significant); * = p ≤ 0.1 (weakly significant)
5. Conclusions:
Many POP were frequently observed in moss samples indicating their suitability to monitor atmospheric deposition of these substance groups by this bioindicator. Challenges exist for PBB, PCB or PFAS either because environmental concentrations are too low with respect to the LOQ or the pollutants’ environmental behavior limit accumulation in moss. It appears to be possible to describe spatio-temporal trends using moss samples. However, due to the low number of sites compared to previous moss monitoring programs for heavy metals and nitrogen and due to present incomplete knowledge of the behavior of the compounds in moss (influence of moss type, eaves effects, site-specific parameters) these investigations still have a rather site-specific character.

6. Acknowledgments:
We are thankful to the German Environment Agency (Umweltbundesamt) for funding this study (FKZ 3720632010). We thank Eurofins GfA LabService for analyzing the samples.

7. References:
Introduction: Pesticides are widely used to control pests in agriculture, but their effects on the environment and human health have raised concerns. Some pesticides have been included in the Stockholm Convention due to their persistence, bioaccumulation, and toxicity (PBT) properties, as well as their ability to undergo long-range atmospheric transport [1]. However, the use of currently-used pesticides (CUPs) has increased in recent years, leading to potential exposure of pesticides, their metabolites and pesticide mixtures in the atmosphere. Pesticides can be transported over long distances from their application sites through various mechanisms, including spray drift, volatilization, and wind erosion [2]. Despite their widespread use, limited information is available on the occurrence, distribution, and transport behaviour of pesticides and their associated metabolites and mixtures in air.

This study investigates the occurrence, distribution and possible off-site transport of pesticides and their metabolites in air at two agricultural regions in Europe (Aveiro District, Portugal and Drenthe, the Netherlands) during a time period of 14 months (April 2021 to June 2022).

Materials and Methods: High-volume air samplers were used to collect 96 air samples, which were analyzed for pesticides in both the gaseous and the particulate phase. PUF/XAD-2 cartridges were employed for sampling pesticides in the gaseous phase, whereas glass-fibre filters (GFFs) were used for the particle phase. The analytical method involved the detection of 319 different pesticides, including organochlorine pesticides, CUPs, and pesticide metabolites. PUF/XAD-2 cartridges were extracted via cold-column extraction with dichloromethane, while the QuEChERS approach was used for the extraction of GFFs. A dispersive solid-phase extraction (d-SPE) was performed for the clean-up of GFFs prior to GC analysis. Instrumental analysis was performed using liquid chromatography coupled to a time-of-flight mass spectrometer (LC-QTOF) and gas chromatography coupled to a tandem mass-spectrometer (GC-MS/MS).

Results: The study detected a total of 96 different pesticides and pesticide metabolites in the air samples collected from the Netherlands and Portugal. These pesticides were present in concentrations ranging from 1.5 pg/m³ to 10 ng/m³ with the highest levels detected during the pesticide application period in spring and summer. The analysis of the particulate phase revealed the presence of 63 pesticides and their metabolites in the Netherlands, and 29 in Portugal. In the gaseous phase, 53 different pesticides were detected in the Netherlands and 24 in Portugal. Pesticides were detected in 85 particulate samples and 93 gaseous samples. More than one pesticide was present in 70 particulate phase samples and 88 gaseous phase samples. Pesticide metabolites were found in 54 of the particulate phase samples and 53 of the gaseous phase samples. For pesticides found in both air phases, the distribution between the gaseous and particle phase was determined by calculating gas-particle partitioning coefficients.

Discussion and Conclusion: The study revealed that pesticide mixtures were present in approximately 70 % of the particulate phase and 90 % of the gaseous phase samples. In addition, pesticide metabolites were detected in more than 50 % of the samples. These results provide important insights into the occurrence and behaviour of pesticides, their metabolites, and mixtures in the atmosphere, and their potential impact on human health.

Acknowledgments: The research leading to these results has received funding from the European Union Horizon 2020 programme under grant agreement n°862568 (SPRINT project, https://sprint-h2020.eu/).

References:
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**Introduction:** The Joint Research Centre of the European Commission has since 2015 produced a series of wet fish tissue Certified Reference Materials (CRMs) for the environmental monitoring of several halogenated organic pollutants (and one CRM for trace metals). These materials are also used for food safety compliance analysis and expand the list of biota CRMs already available in the JRC catalogue for the monitoring of halogenated POPs. This contribution, while giving a summarised overview, will focus more in detail on the last additions to this series: ERM-CE100, first ever matrix RM certified for chlorinated paraffins (CPs) and ERM-CE103 (close to finalisation).

**Materials and Methods:** The starting materials used for producing the CRMs were naturally contaminated Wels catfish (*Silurus glanis*), pike perch (*Lucioperca lucioperca*), trout (*Salmo trutta*) and Nile perch (*Lates niloticus*). The innovative processing approach for obtaining the CRM matrix in a wet form used, in brief, pre-cooking and autoclaving at 121 °C to ensure stability. For ERM-CE100, ERM-CE102 and ERM-CE103, the processing step involved the mixing with other blank fish species to meet appropriate levels of the target analytes for the certification. The certification was performed in accordance to ISO 17034:2016 and ISO Guide 35:2017. The materials were value-assigned by an intercomparison of laboratories of demonstrated competence, adhering to ISO/IEC 17025:2017.

**Results:**

**Discussion and Conclusion:** With the exception of some NIST SRMs in a frozen form (that have to be stored at - 80 °C), most biota CRMs commercially available for the analysis of contaminants are in a lyophilised form, i.e. dry powders. The initial drive to develop CRMs in a wet matrix, baby-food like paste (stable at + 4 °C), was the setting of biota Environmental Quality Standards (EQS) on wet weight basis for the monitoring of priority substances under the EU Water Framework Directive. Additionally, such a wet matrix enhances the commutability (i.e., representativeness) of CRMs by closer mimicking routinely analysed samples and simplifies the compliance check against the EQSs. The certification of short-chain and medium-chain chlorinated paraffins (SCCPs and MCCPs, respectively) was finalised in December 2022 in the fish tissue ERM-CE100, already available in the JRC catalogue with certified values for hexachlorobenzene (HCB) and hexachlorobutadiene (HCBD) [1]. CPs certified values are in the range of 20 to 50 μg/kg (wet weight), with expanded uncertainties of about 30 % for SCCPs and 39 % for MCCPs, reflecting the known difficulty in obtaining accurate analytical results for these analytes. This certification was part of the Eurostars project Chloffin that delivered also an enlarged suite of pure substance CRMs and improved methods for CPs analysis [2].

ERM-CE103 is planned for release before end 2023 with certified values of about 20 μg/kg (wet weight) for pentachlorobenzene (PeCB) and between 0.2 and 0.5 μg/kg (wet weight) for hexachlorocyclohexanes (HCHs). The low levels of HCHs (and the heavily polluted matrix) have been a challenge for the laboratories participating to the CRM characterisation campaign. The HCHs pattern in ERM-CE103 reflects the shift observed during the last decades among the isomers, with an increased presence of β-HCH (compared to α-HCH and γ-HCH), due its persistency and higher bioaccumulative properties [3].

**Acknowledgments:** The authors would like to thank S. Choquette (NIST, US) for the provision of SRMs as quality control samples for the certification and J. Seghers (EC-JRC) for the development of the materials’ processing method.

**References:**
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1. Introduction:
Polycyclic aromatic hydrocarbons (PAHs) and their halogenated derivatives (XPAHs) are emitted by anthropogenic activities with incomplete combustion. The PAHs and XPAHs are partitioned between gaseous and particulate phases. Some PAHs and XPAHs have adverse effects for human health such as carcinogenicity and mutagenicity. The measurements of individual exposures to PAHs and XPAHs are needed to assess their human health risks. Inhalation exposure is estimated using air concentrations of these chemicals. The concentrations of PAHs and XPAHs in air are calculated from their amounts collected with mini pump carried by individuals ¹. However, the mini pumps are noisy and requiring electric power. In addition, requirement to carry the mini pump during the study influences participants’ behavior ². A recent study showed that silicone wristband could be a useful tool as passive sampler to measure individual exposures to PAHs because of no requirement of electric power ³. However, previous studies showed the collection capabilities of mainly USEPA 16 PAHs by silicone wristbands, and there is no information about the collection capabilities of XPAHs by passive sampling method using silicone wristbands. In addition, passive samplers were affected by meteorological conditions (e.g., wind speed) ⁴, and there is no information about the effects of meteorological conditions to silicone wristbands.

The aims of this study were: (1) to evaluate the relationships between the concentrations of PAHs and XPAHs measured by active sampling and their collection amounts by passive sampling using silicone wristbands; (2) to assess the effects of distribution phase for PAHs and XPAHs to sampling rate; (3) to assess the wind effects during the sampling.

2. Materials and Methods:
Before sampling, the silicone wristband was pre-washed with 250 mL of dichloromethane for 24 h in a Soxhlet extractor and dried in a desiccator. After sampling, the silicone wristband was shredded, spiked with 2 ng of each recovery standard (Phe-²¹C₆, Flu-²¹C₆, Chr-²¹C₆, BaP-²¹C₄, 1-ClPyr-²¹C₆, 7-ClBaA-²¹C₆, 7,12-Cl₂BaA-²¹C₆, and 7-BrBaA-²¹C₆), and then Soxhlet- extracted with 250 mL of dichloromethane for 16 h. The extract after concentration was loaded on an activated carbon cartridge (Carboxen 1016, 200 mg, Supelco, St. Louis, MO, USA) and a silica gel cartridge (Supelclean LC-Si, 2 g, Supelco) connected in series, and 20 mL of 10% dichloromethane/hexane were added for removing matrices from the cartridges. The silica gel cartridge was then removed, and the activated carbon cartridge was reversed and eluted with 180 mL of toluene. The toluene fraction containing PAHs and XPAHs was evaporated and transferred to a vial. The solvent then was changed to 100 µL of isooctane, and the extract was spiked with 2 ng of each internal standard (Phe-²⁰d₁₀, Flu-²⁰d₁₀, and BaP-²²d₁₂) in isooctane, and analyzed for PAHs and XPAHs.

Active sampling using mini pump was simultaneously carried out to determine the concentrations of target chemicals in air. The adsorbent (ORBO-42, 66/33 mg, Supelco) and the filter TF98 (25 mm diameter, Sibata Scientific Technology Inc.) were connected in series and attached to a mini pump MP-WSP (Sibata Scientific Technology Inc.). The adsorbent was extracted with 4 mL of dichloromethane; the filter was Soxhlet-extracted with 250 mL of dichloromethane for 16 h. These extracts were analyzed by using a gas chromatograph-triple quadrupole mass spectrometer (GC 7890B-MS 7010B, Agilent Technologies). The purification processes during recovery tests using analytical standards achieved favorable recovery rates (61%–118%) of target PAHs and XPAHs ⁵. In addition, acceptable recovery rates of the internal standards (55%–92%) were obtained from all samples in this study.

Figure 1: Silicone wristband as a passive sampler.
3. Results and Discussion:

Comparison of concentrations by active sampling with their collection amounts by passive sampling using silicone wristbands:

All PAHs and XPAHs were detected at least one silicone wristbands. Previous studies showed the collection capabilities of mainly USEPA 16 PAHs by silicone wristbands, and there is no information about the collection capabilities of XPAHs by passive sampling method using silicone wristbands. This study, for the first time, provides comprehensive information on the collection capabilities of a diversity of PAHs and XPAHs compared with previous studies by passive sampling method using silicone wristbands. Although the range of collection amounts of PAHs in previous studies were within 2 orders of magnitude, those in this study were within 2−6 orders of magnitude.

The comparison of anthracene (Ant) and benzo[a]pyrene (BaP) concentrations by active sampling (C_{AS}) with their collection amounts by passive sampling (W_{PS}) are shown in Figure 2. The C_{AS} showed statistically significant positive correlations with the W_{PS} for both Ant and BaP (p<0.01), although Ant and BaP distribute in gaseous and particulate phase, respectively. For 24 PAHs and 27 XPAHs, the C_{AS} also showed statistically significant positive correlations with the W_{PS} (p<0.05). The air concentrations of these chemicals distributed in both gaseous and particulate phases could be therefore estimated from their collection amounts by silicone wristbands. In contrast, there were no significant correlations between C_{AS} and W_{PS} for high molecular PAHs and some of XPAHs. Because high molecular PAHs and some of XPAHs occurred at low concentrations in the environment, only limited information on the concentrations for these chemicals was available in this study.

Comparison of sampling rates of gaseous and particulate PAHs and XPAHs: The comparison of sampling rates between gaseous and particulate PAHs/XPAHs are shown in Figure 3. The sampling rates (SR) were calculated according to the following equation:

\[ SR = \frac{W_{PS}}{(C_{AS} \cdot t)} \]

where W_{PS} is the collection amounts by passive sampling (pg), C_{AS} is the concentrations by active sampling (pg L^{-1}), and t is sampling duration (min).

The sampling rates of chemicals targeted in this study were 0.0500−25.4 L min^{-1} WB^{-1} (median, 1.31 L min^{-1} WB^{-1}). Among other passive samplers described in previous studies, sampling rates of PAHs using XAD-4 resin tube and PUF passive samplers were 0.0417−0.972 L min^{-1} and 3.47±2.50 L min^{-1}, respectively. Sedlackova et al. estimated sampling rates of SVOCs (e.g., PAHs, poly chlorinated biphenyls (PCBs), poly brominated biphenyls (PBDEs), and organochlorine pesticides (OCPs)) with silicone sheet (0.5 mm thick, total surface area were 300 cm²) by using a model based on mass transfer theory, and their sampling rates were 0.466−0.567 L min^{-1}. The sampling rates of silicone wristbands in this study were comparable to and/or higher than the other passive samplers in previous studies. The silicone wristbands proposed in this study are thus applicable as passive samplers.

The sampling rates of gaseous PAHs and XPAHs were significantly higher than those of particulate PAHs and XPAHs (Welch’s t-test: p<0.01). In a previous study, the sampling mechanism of passive samplers for SVOCs, which was distributed in gaseous phases, were discussed based on the two-film theory. The SVOCs are resisted by the air-boundary layer and the sampler-
A Passive Sampling Method Using Silicone Wristband for Determination of Polycyclic Aromatic Hydrocarbons and Their Halogenated Derivatives

boundary layer and collected into the sampler by molecular diffusion as a driving force. In contrast, the collection amounts of particle by passive sampling depended on physical mechanisms of entrainment including diffusion, impaction, interception, and sedimentation. Although there is no information about the sampling mechanism of silicone wristbands, the sampling mechanism of silicone wristbands could be similar to other passive samplers. These findings suggest that the difference of sampling rates between gaseous and particulate phases is attributed to each sampling mechanism.

Effects of wind speed to sampling rates of PAHs and XPAHs: The comparison of phenanthrene (Phe) and chrysene (Chr) collection amounts by silicone wristbands under different wind speed conditions are shown in Figure 3. Although Phe and Chr distribute in gaseous and particulate phases, respectively, the collection amounts significantly increased with increasing wind speed in both chemicals (Welch’s t-test: *p<0.01). The other target gaseous and particulate PAHs/XPAHs showed similar tendencies. Harner et al. reported that collection amounts by PUF used as passive sampler increased with increasing wind speed. Higher wind speed has the effect of increasing molecular diffusion rates based on Fick’s first law by diminishing the air-boundary layer surrounding the sampler. Limited information is available for wind effects on silicone wristbands. Tromp et al. reported that sampling rates of silicone wristband measured in the chamber experiments were higher than those observed from the field studies, because of the high air velocity of 1.3 m s⁻¹ in the chamber. These results suggest that the sampling mechanism of gaseous PAHs and XPAHs by silicone wristbands is similar to that of PUF. In addition, higher wind speed has the effect of increasing frequency of particle impaction to passive samplers. The increase of collection amounts for particulate chemicals observed in this study could be attributed to the increasing frequency of particle impaction. These suggest that molecular diffusion for gaseous chemicals and frequency of impaction for particulate chemicals were affected by wind speed, and consequently, the sampling rates of PAHs and XPAHs increased with increasing wind speed.
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4 Conclusions:
In this study, we revealed that silicone wristbands enable to collect the PAHs and XPAHs with a wide range of concentrations compared with previous studies. In addition, we showed that the concentrations of 24 PAHs and 27 XPAHs in air could be estimated from their collection amounts in silicone wristbands. There is limited information about the estimation of other SVOC concentrations in air from their collection amounts by silicone wristbands. Further studies about the relationships between the concentrations in air and the collection amounts in silicone wristbands for other SVOCs are needed.

We suggest that the difference of sampling rates between gaseous and particulate is attributed to each sampling mechanism. We also suggest that the sampling rates of PAHs and XPAHs increase with increasing wind speed because of increasing the molecular diffusion rates and the frequency of particle impaction. However, there is a lack of sample number and sampling condition. Further studies are needed to evaluate the effects of wind speed on the sampling rates of silicone wristbands.

5 Acknowledgments:
This study was supported by a Grand-in-Aid for Scientific Research (B) from the Japan Society for the Promotion of Science (No. JP21H03614) and the Environment Research and Technology Development Fund (JPMEERF18S11704, JPMEERF20231M04) of the Environmental Restoration and Conservation Agency of Japan.

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Fingerprinting of CPs and their Olefinic Transformation Products in Electronic Plastics of the Swiss Market

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10:00 - 12:00
Progress in Methods for POPs Analysis

Chairs: S.Yin & J.Stubleski

WED-AM-D2

1. Introduction:
Chlorinated paraffins (CPs) are high-production volume chemicals widely used in plastics. They can be described with the molecular formula C_{n+2}H_{2n+2}Cl_{x}, where C-homologues differ in their carbon number (n) and Cl-homologues in their chlorine number (x). CPs can also be classified based on their carbon-chain length as short-chain (SC, C1–C3), medium-chain (MC, C4–C13) and long-chain (LC, C14–C21) CPs. [1] Herein, we further classified very short-chain (vSC, C<10) and very long-chain (vLC, C≥22) CPs. Technical CP-mixtures may contain chlorine between 30 to 70% by mass. This leads to intricate mixtures with hundreds of carbon- (C, n = 9–34) and chlorine- (Cl, n = 2–26) homologues. CPs are commonly used as plasticizers, flame-retardants and coolant fluids. Due to that, CPs can be found in various plastic materials, electronic devices and plastic consumer products. Since 2017, SCCPs are listed as persistent organic pollutants (POPs) by the Stockholm Convention. Nowadays, MCPPs are classified as substances of concern too and are under revision. Exposing CPs to heat favors consecutive HCl-losses forming chlorinated mono- (COs), di- (CdiOs) and tri- (CtriOs) olefins. [2] This leads to additional hundreds of olefinic transformation products. CPs and their transformation products were analyzed by RP-LC-APCI-Orbitrap-MS as their singly charged chloride-adduct ions [M+Cl]. The resulting complex mass spectra were evaluated with an R-based Automatic Spectra Evaluation Routine (RASER) introduced by Knobloch et al. [3] Recently, a fingerprinting method of CPs was established to evaluate complex homologue data to distinguish between CP-containing items. [4] It is based on the multidimensional analysis of patterns formed by hundreds of C- and Cl-homologues of CPs, COs, CdiOs and CtriOs. Such patterns reveal features, which are characteristic of the CP-mixtures (and side products) that constitute the plastic item and can be used to characterize them unequivocally. We hypothesized that plastic materials from the Swiss market contain CPs and their transformation products and can be distinguished by their fingerprints.

2. Materials and Methods:
A black jumper cable (P1) and an LED stripe (P2) were collected from the Swiss market by Cantonal laboratories and the Swiss Federal Office for the Environment. CPs and their transformation products were extracted by Soxhlet with dichloromethane (60 mL, 40 °C, 4 h) and concentrated to 20 mL. Aliquots were spiked with 13C-isotopically labelled 1,5,5,6,6,10-hexachlorodecane. Dissolved plastic was precipitated by adding MeOH until a 1:1 proportion was achieved and purified by normal-phase chromatography. CP-containing fractions were concentrated, dissolved in MeOH and analyzed by high-resolution RP-LC-APCI-Orbitrap-MS (12 Hz scanning frequency, R=120000). MS data of singly charged chloride-adduct ions [M+Cl] in a range of m/z from 100 to 1100 were semi-automatically processed with RASER. C-/Cl-homologue distributions were acquired by comparing experimental and simulated isotopic patterns. Fingerprints based on carbon-chain lengths, chlorination and saturation degrees were obtained for CPs, COs, CdiOs and CtriOs by applying reported methods.

3. Results and Discussion:
Carbon- and chlorine-homologue distributions of CPs, COs, CdiOs and CtriOs were obtained after evaluating respective mass spectra with RASER (Figure 1). In sample P1, 16 C-homologues (C 9–C24) and 14 Cl-homologues (Cl 3–Cl16) were found for CPs. Olefinic material such as the COs (C 9–C23 and Cl3–Cl11), CdiOs (C10–C22 and Cl3–Cl10) and CtriOs (C13–C22 and Cl4–Cl9) were identified next to CPs. Relative abundance of CPs in P1 was 91%, whereas those of COs, CdiOs and CtriOs were 7%, 1% and <1%, respectively. Similarly, abundances of CPs, COs, CdiOs and CtriOs in P2 were 90%, 8%, 1% and <1%, respectively. C-/Cl-homologue distributions of CPs and COs showed similar shapes both for P1 and P2. Respective distributions of CdiOs and CtriOs displayed analogous trends too, with fewer C- and Cl-homologues detected. In accordance with sample P1, the C0-homologue distribution in P2 showed similarities with the one of CPs, albeit fewer C-/Cl-homologues were detected. Particularly, respective C-/Cl-homologue distributions of CdiOs and CtriOs did differ substantially from the ones of CPs and COs. In P1, C_{14}Cl_{14}-, C_{14}Cl_{13}-, C_{13}Cl_{13}- and C_{13}Cl_{12}-homologues were the most abundant (MAH) for CPs, COs, CdiOs and CtriOs, respectively. In P2, C_{14}Cl_{14}-, C_{14}Cl_{13}-, C_{13}Cl_{13}- and C_{13}Cl_{12}-homologues were the most abundant (Figure 1). Comparable homologue distributions were reported previously for other plastic consumer products showing unimodal C- and Cl-homologue distributions. [1,4] The homologue distribution of P2 can be compared to a previously reported garden cloche, whereas the one of P1 was not observed before. Earlier studies reported until Cl_{14}-homologues, but herein, Cl_{15}- and Cl_{16}-homologues were found as well.
Therefore, these plots highlight the large variability of CP-, CO-, CdiO- and CtriO-distributions in plastic items.

Fingerprints from CPs and their transformation products were obtained for electronic plastic samples P1 and P2 (Figure 2). Such fingerprints were deduced from MI\textsubscript{100\%} data and are based on the saturation degree (A), carbon-chain length (B and E) and chlorination degree (C and D).

As shown in Figure 2 (A), CPs (blue) were the most abundant class with respect to the olefinic material in both samples. However, the relative olefinic content varied throughout the C-homologues. Sample P1 (A1) showed a bimodal distribution with maxima of olefinic material for C\textsubscript{18} and C\textsubscript{22}-homologues, whereas the distribution of P2 (A2) was unimodal with a maximum of olefinic material for C\textsubscript{17}-homologues. In both cases, proportions of olefinic material decreased for the longest homologues. Similar trends with distinct maxima were reported previously.\textsuperscript{[4]} However, the high variability of such mixtures impede a total match with previous studies. In any case, both plastic items could be distinguished by their paraffin- and olefin-proportions.

The proportions of different carbon-chain length classes for CPs in plastic P1 (B1) showed considerable contributions of SCCPs (41%) and MCCPs (49%) with traces of vSCCPs (1%), LCCPs (8%) and vLCCPs (<0.1%). On the other hand, sample P2 (B2) mainly consisted of SCCPs (44%) and MCCPs (55%) with low relative abundances of vSCCPs (<0.01%), LCCPs (<1%) and vLCCPs (<0.01%). A trend towards larger proportions of longer-chain material for COs, CdiOs and CtriOs was observed in both samples. These plots match to a certain degree the one from a yoga mat reported previously.\textsuperscript{[4]} Despite such similarities the proportions of carbon-chain length classes can be used to discriminate between P1, P2 and the mentioned yoga mat.

The proportions of different chlorine-homologues per C-homologue can be calculated (Figure 2, C). In P1 (C1), shorter-chain homologues (C\textsubscript{9}–C\textsubscript{15}) contained mainly lower-chlorinated material (Cl\textsubscript{4}–Cl\textsubscript{7}), whereas longer-chain homologues (C\textsubscript{16}–C\textsubscript{24}) also contained around 40% of higher-chlorinated material (Cl\textsubscript{8}–Cl\textsubscript{16}). The relative abundances of Cl-homologues varied with the chain-length highlighting a bimodal distribution. The point of inflection was found at C\textsubscript{20}-homologues. On the other hand, plastic P2 (C2) showed a unimodal distribution where lower-chlorinated homologues (Cl\textsubscript{3}–Cl\textsubscript{7}) became gradually less predominant towards longer chain material (C\textsubscript{17}–C\textsubscript{25}). Although uni- and bi-modal patterns were reported before, the specific pattern of different Cl-homologues and the trends did not match previous findings exactly. Hence, the Cl-homologue distributions constitute another important characteristics of the fingerprint of plastic items.

Mean chlorine numbers (n\textsubscript{Cl}) determined per C-homologue in P1 (D1) showed a gradual increase from n\textsubscript{Cl} = 6.19 (C\textsubscript{9}) until 6.91 (C\textsubscript{24}) with a point of inflection at C\textsubscript{20} of 7.11. The weighted mean chlorine number (red line) for P1 was 6.86. In P2 (D2), n\textsubscript{Cl} increased steadily throughout the C-homologues from 6.06 (C\textsubscript{9}) until 9.48 (C\textsubscript{25}) with a weighted mean chlorine number (red line) of 7.02. The n\textsubscript{Cl} trend was comparable with the ones reported before for the Cl-homologue distributions (Figure 2, C). We conclude that n\textsubscript{Cl}-values did not match for P1 and P2. However, n\textsubscript{Cl} is another important criterion of the fingerprint.
Mean carbon numbers ($n_C$) computed per chlorine-homologue in P1 (E1) showed a convex shape with $n_C$-values varying from 18.30 (Cl$_3$) to 19.27 (Cl$_{16}$) and a minimum at Cl$_6$ of 13.32. The weighted mean carbon number (red line) in P1 was 13.97. Similarly, $n_C$-values of P2 (E2) showed a convex shape too but with lower values such as 14.31 (Cl$_4$) and 16.76 (Cl$_{14}$) and a minimum at Cl$_6$ of 13.20. Both, the $n_C$-values and the trends did not match previous studies. Therefore, the $n_C$- as well as the $n_Cl$-criteria could be used to discern between P1 and P2 (Figure 2, D and E).

**4 Conclusions:**
Two electronic plastic items collected from the Swiss market in 2021 were successfully analyzed by RP-LC-APCI-Orbitrap-MS and the corresponding mass spectra were efficiently evaluated with RASER. Characteristic C- and Cl-homologue distributions were obtained for CPs, COs, CdiOs and CtriOs. Fingerprints based on the saturation degree, carbon-chain length and chlorination degree were then deduced from homologue distributions. The analysis of such patterns could distinguish between P1, a black jumper cable and P2, an LED stripe. These findings can now be compared with previous studies. None of the samples was ambiguous. Therefore, the fingerprinting method has the potential to discern between multiple plastic items, which can contain diverse CP-mixtures of hundreds of C- and Cl-homologues.

**Figure 2:** Characteristic fingerprints of plastic samples P1 (jumper cable, top) and P2 (LED stripe, bottom). The following plots are displayed: paraffinic vs. olefinic proportions (A), proportions of carbon-chain length classes (B), chlorine-homologue distributions (C), mean chlorine numbers ($n_{Cl}$, D) and mean carbon numbers ($n_C$, E). Weighted mean chlorine and carbon numbers (red lines in D and E) are depicted.
WED-AM-D2  Fingerprinting of CPs and their Olefinic Transformation Products in Electronic Plastics of the Swiss Market

Acknowledgments:
This work was supported by the Swiss Federal Office for the Environment (FOEN). Samples studied in this work were provided by FOEN and Cantonal Laboratories.

References:
Progress in Methods for POPs Analysis

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1. Introduction:
Polycyclic aromatic hydrocarbons (PAHs) are a group of organic compounds containing two or more fused aromatic rings. These environmental pollutants are ubiquitous and originate from the incomplete combustion of organic matter and/or mobilization by micro-organisms. Due to their hydrophobic nature, they tend to accumulate in sediments and soils, as well as in the tissues of living organisms, including humans (Goldman et al., 2001; Li et al., 2010). Meanwhile, PAHs in water are frequently detected as well (Li et al., 2010). PAHs are known to be harmful to human health and the environment, with some of them being carcinogenic and mutagenic (Goldman et al., 2001). To assess the potential toxicity of PAHs, 16 EPA PAHs are commonly monitored using instrumental analytical methods such as gas chromatography-mass spectrometry (GC-MS). While it is a highly sensitive method, it has some limitations when it comes to assessing the bio-reactivity of PAHs and their mixtures. This is because PAHs are often present in complex mixtures, and the toxicity of these mixtures is difficult to predict based on the individual components alone (Andersson and Achten, 2015). In addition, derivative forms of PAHs, such as methylated PAHs (Me-PAHs), are also of growing concern since they are frequently detected in the environment and have been shown to be more toxic than their parent compounds (Larsson et al., 2018, 2014).

To address these challenges, the Chemically Activated LUCiferase gene eXpression (CALUX) bioassay was developed to screen for activation of the aryl hydrocarbon receptor (AhR) signaling pathway, which is a well-known receptor for polycyclic aromatic compounds (He et al., 2011; Pieterse et al., 2013). This bioassay consists of recombinant cell lines that have been stably transfected with AhR-responsive luciferase reporter genes (Murk et al., 1996). Upon exposure to AhR agonists, the activation of AhR leads to the production of luciferase, which is proportional to the amount and potency of the ligands that are present in the sample to which the cells are exposed (Murk et al., 1996).

Although interactions between PAHs and AhR have been investigated previously using the CALUX cell line H4IIe cells (Larsson et al., 2014; Pieterse et al., 2013), few studies have assessed the relative potencies (REP) of Me-PAHs (see however Boonen et al., 2020). The aim of this study is to optimize the CALUX assay based on H1L7.5c1 cell lines (He et al., 2011) using benzo[a]pyrene (BaP) as a reference ligand and to assess the AhR-induced activity of sixteen EPA priority PAHs and five Me-PAHs individually and in the mixture. A growing body of experimental evidence indicates that the in vitro activities of these mixtures could be predicted from the overall response of their components using the concept of the concentration addition (CA) model and/or the independent mode of action (IA). Both of these models assume an additive effect, i.e. the chemicals in the mixture act together but do not interact with each other. This is the hypothesis we tested in this study with mixtures of 2-5 constituents including PAHs and Me-PAHs.

2. Materials and Methods:
The PAHs, including naphthalene (Nap), acenaphthylene (Acy), acenaphthene (Ace), fluorene (Flu), phenanthrene (Phe), anthracene (Ant), fluoranthene (Fla), pyrene (Pyr), chrysene (Chr), benzo[a]anthracene (BaA), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[a]pyrene (BaP), dibenzo[a,h]anthracene (DahA), benzo[ghi]perylene (BghiP), and indeno[1,2,3-cd]pyrene (IcdP), as well as methylated PAHs, including 2-methylanthanthrene (2-MNap), 5-methylchrysene (5-MChr), 1,3-dimethylanthanthrene (1,3-DMNap), 9,10-dimethylnaphthalene (9,10-DMNap), 9,10-dimethylnaphthalene (9,10-DMAnt), 7-methylbenzo[a]pyrene (7-MBap), were all purchased from Sigma-Aldrich (Germany). The working solutions were diluted in dimethyl sulfoxide (DMSO, ≥ 99.7%, Sigma-Aldrich, Germany).

The cell cultural reagents were all purchased from Gibco by Life Technologies (the United Kingdom). The working alpha-Minimum Essential Medium (α-MEM) was supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (pen-strep). The working Dulbecco’s Modified Eagle Medium (DMEM, without phenol red) was supplemented with 5% charcoal-stripped FBS, 2% l-glutamine (200 mM), 1% sodium pyruvate (100 mM, sterile-filtered), and 1% pen-strep. Trypsin (with and without phenol red, 10×, 0.5%) and phosphate buffered saline (PBS, 10×, pH 7.4) were diluted to 1× as working solution. Luciferin and lysis reagent were homemade.

With regard to the AhR-CALUX bioassay, H1L7.5c1 recombinant mouse hepatoma cell lines were used. In general, cells were cultured in working α-MEM and maintained in an incubator at 37 °C, 5% CO2, and 80% humidity. The cells were seeded into 96-well plates at a density of 30,000 cells/well in 150 µL of growth medium and allowed to incubate for 48 h prior to dosing.
Next, 100 µL of reclaimed media containing the desired concentration of the testing compounds were added to each well in triplicate, resulting in a final concentration of 1% (v/v) DMSO. After incubation for 2.75 h, the cells were washed with 100 µL of PBS buffer and assessed for dead cells with a microscope before being lysed with 50 µL of lysis buffer. Luciferase activity was measured using a luminometer (Tristar Reader, Berthold Technologies) with 50 µL of luciferin added automatically. The light output was measured as relative light units (RLUs) after a counting time of 3 s. Medium blank and DMSO solvent blank were included on each plate as quality controls. The resulting RLUs were fitted to a four-parameter Hill equation according to Elskens et al. (2011).

3. Results:
The reliability of the assay was first examined by analyzing the BaP calibration standards (n = 9) repeatedly. An example of BaP calibration curve is shown in Figure 1. The limit of detection (LOD), defined as 10% of maximum response, was found to be 0.4 ± 0.1 pg/well, while the limit of quantification (LOQ), defined as 20% of maximum response, was determined to be 2.8 ± 0.8 pg/well. A positive control (PC, 47 pg BaP/well) was measured in triplicate in each plate to monitor the quality of the calibration curve. The coefficient of variance (CV) of intra-laboratory repeatability was 15.5%, and CV of intra-laboratory reproducibility was 13.2%. The accuracy of the assay was expressed as percent error of measured PC, ranging from −11.5 to 11.6% with an average of 5.1%.

The agonistic activities of sixteen EPA PAHs and five Me-PAHs were tested using PAH-CALUX. Among these chemicals, Ace, Flu, Nap, Phe, 1,3-DMNap, and 2-MNap did not produce any response. It suggests that the absence of activation of the parent PAHs on the AhR may lead to the lack of response of their corresponding Me-PAHs on the same receptor. However, it should be noted that this conclusion does not apply to the entire PAC family, as 9,10-DMAnt was also identified as a non-AhR agonist, while Ant was reported as a weak AhR agonist in this study.

The relative potency (REP) is a measure of the potency of a chemical relative to a reference chemical, typically expressed as EC50 value of a thoroughly characterized standard (BaP in this study) by that of a sample or pure compound. The results of REPs showed that BkF was the most potent PAC congener with a relative potency of 7.2, followed by DahA > BbF > IcdP > 5-MChr > 7-MBaP were all higher than BaP (Table 1). It suggests that these compounds have the potential to pose a greater risk to human health. It is interesting to note that some of the compounds with higher REP values, such as 7-MBaP and 5-MChr, are methylated forms of BaP, suggesting that the addition of a methyl group can increase the potency of these compounds.

It is important to note that the assessment of potency and efficacy solely based on REP values can be intricate because their evaluation can vary with target receptors or enzymes in different cell species. In addition, REP is only applicable if the dose-response curves for the test compounds and standard are parallel (i.e., with the same Hill coefficient) and possess the same efficacy (i.e., maximum achievable response). But these conditions are often either violated or cannot be demonstrated. Consequently, the inclusion of maximum response, Hill coefficients, and EC50 in our results is also listed to have a better understanding of the characteristics of these compounds (Table 1).
Table 1: Dose-Response curve parameters for PAHs and their derivatives. All experiments were performed in 3 independent experiments with triplicate measurements of each tested concentration on one plate, and the data were expressed as mean ± standard deviation (SD).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Max. RLU (%)</th>
<th>Hill coefficient</th>
<th>EC50 (pg/well)</th>
<th>REP50</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-Methylchrysene</td>
<td>97.7 ± 3.3</td>
<td>1.0 ± 0.4</td>
<td>14.8 ± 1.2</td>
<td>2.4 ± 0.4</td>
</tr>
<tr>
<td>7-Methylbenzo[a]pyrene</td>
<td>97.1 ± 3.2</td>
<td>0.8 ± 0.2</td>
<td>23.6 ± 1.0</td>
<td>1.2</td>
</tr>
<tr>
<td>Acenaphthylene</td>
<td>70.0 ± 4.4 **</td>
<td>1.3</td>
<td>(39.9 ± 1.5) × 10^4</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Anthracene</td>
<td>76.9 ± 1.3 **</td>
<td>1.1 ± 0.1</td>
<td>(153.6 ± 30.5) × 10^2</td>
<td>0.002</td>
</tr>
<tr>
<td>Benz[a]anthracene</td>
<td>94.6 ± 1.3</td>
<td>0.8 ± 0.1</td>
<td>49.6 ± 4.7</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>96.5 ± 0.2</td>
<td>0.9 ± 0.2</td>
<td>28.2 ± 7.2</td>
<td>1.00</td>
</tr>
<tr>
<td>Benzo[b]fluoranthene</td>
<td>98.2 ± 2.9</td>
<td>0.7 ± 0.1</td>
<td>10.8 ± 3.7</td>
<td>3.3 ± 0.3</td>
</tr>
<tr>
<td>Benzo[ghi]perylene</td>
<td>78.56 ± 0.6 *</td>
<td>1.0 ± 0.1</td>
<td>(2.9 ± 0.2) × 10^3</td>
<td>0.01</td>
</tr>
<tr>
<td>Benzo[k]fluoranthene</td>
<td>106.2 ± 4.7</td>
<td>0.7 ± 0.1</td>
<td>4.0 ± 0.6</td>
<td>7.2 ± 1.2</td>
</tr>
<tr>
<td>Chrysene</td>
<td>100.1 ± 2.3</td>
<td>1.0 ± 0.1</td>
<td>30.3 ± 9.1</td>
<td>1.00 ± 0.4</td>
</tr>
<tr>
<td>Dibenzo[a,h]anthracene</td>
<td>101.2 ± 1.2</td>
<td>0.6 ± 0.1</td>
<td>7.1 ± 0.7</td>
<td>4.0 ± 0.4</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>34.0 ± 2.7 **</td>
<td>2.4 ± 0.6 **</td>
<td>(8.4 ± 2.8) × 10^3</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Indeno[1,2,3-cd]pyrene</td>
<td>97.8</td>
<td>0.9 ± 0.1</td>
<td>11.2 ± 2.1</td>
<td>2.6 ± 0.5</td>
</tr>
<tr>
<td>Pyrene</td>
<td>11.2 ± 1.4 **</td>
<td>1.4 ± 1.2 **</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
</tbody>
</table>

n.a.: not available

*p<0.05, **p<0.01 (p-values are in reference to the comparison with BaP)

4. Discussion:
According to our knowledge, this was the first study to test the AhR-activity of 7-MBaP, 1,3-DMNap, 2-MNap, and 9,10-DMAnt. By comparing the REP values, this study demonstrated that BkF, DahA, BbF, and IcdP were more potent AhR agonists than BaP, which is consistent with previous findings from Pieterse et al. (2013). However, the order of potency among the PAHs differed between both studies. In accordance with this study, Pieterse et al. (2013) also reported that Ace, Acy, Ant, Flia, Flu, Nap, and Phe were weak or non-agonists in their assay. Moreover, they reported REPs of 5-MChr and Chr to be 1.4 and 0.8, respectively, which were lower than those found in this study. However, the finding that methylated form is more potent than its parent remained the same. Differences in the detection of AhR agonist activity for some PAHs were also observed. For example, Amakura et al. (2016) found weak AhR agonist activity for Flu, which was not observed in our studies. These findings suggest that the choice of cell line and experimental parameters can impact the detection of AhR agonist activity for PACs. For the experiment with mixtures, in 95% of the cases, the observed pattern was a joint and independent effect of PAHs and Me-PAHs on the AhR. Overall, these results validate a risk assessment approach based on an additive effect model possibly modulated by intrinsic toxicity factors.

5. Conclusions:
This study presents a rapid, reliable, sensitive, and accurate method for screening the activity of PACs. The agonist activity of sixteen PAHs and 5 methylated derivatives was determined with twelve of them showing strong agonistic activity. Future analysis of antagonistic activity will provide more insight and information about mixture effects.
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6. Acknowledgments:
Y. Su was supported by the China Scholarship Council (CSC), grant No. 202107650006.

7. References:
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Introduction:
Persistent organic pollutants (POPs) like PCDD/F, PCBs, PBDEs and PCNs are toxic chemicals that persist in the environment and accumulate in the food chain. They are often referred to as "forever chemicals" because they do not break down and remain in the environment for many years. To address the problem of POPs in food, feed, and the environment, international agreements, such as the Stockholm Convention on POPs, have been established to regulate the production, use, and disposal of these chemicals. The goal is to reduce exposure to POPs and prevent further harm to human health and the environment. These policies have lead to an increase in samples number that are analyzed for various POPs and therefore crated the need for laboratories to come up with strategies to shift from a manual sample preparation to automated strategies. Automation of the clean-up process in the analysis of various POPs offers several advantages, including improved accuracy, precision and efficiency, as well as reduced sample preparation times with higher sample throughput. Additionally, it has become more and more important to develop methods with which several analytes can be cleaned up in one simple run.

Material and Method:
Various environmental as well as food and feed sample were extracted with pressurized fluid extraction (PFE). In contrast to other commonly known extraction systems for pressurized extraction, the system applied is extracting the samples in a pressure range of max 17 bar and result in excellent recoveries at very short runtimes. Further advantages of this pressure range selected result in design benefits of the equipment e.g. bigger dimensions of various tubes can be employed leading to less risk of clogging, lower wear and tear of the material, which in total bring a more reliable application and less down time of the instrument. Extractions times vary depending on the method and sample from 15- 30 min. For the multimethod clean up, to cover in the fractions PCBs, PBDEs, PCDD/Fs und PCNs, an established automated cleanup unit DEXTech was applied. The dedicated fractionations were collected after fat digestion on acidic silica column with different capacities of 1g, 5g and up to 8g of fat. These were combined with an alumina oxide and a carbon column clean up. Depending on the samples process times from 45 min up to 65min are due. Additionally to the multi method described, a variety of methods for single POPs clean up, like Dioxin only, PCB only Method or all 209 PCBs method are also available, to fulfill the application as needed for individual tasks or pressure in a laboratory e.g. using two columns only or running with shorter process times. All concentrations and solvent exchange steps were performed with a special vacuum centrifuge with dedicated pressure profile and a specially added stop sensor, to save the samples from over boiling or loosing of analytes. As the end volumes in the evaporation process are sensor controlled, the process doesn’t need an supervision.

Results:
In this talk the multi methods and results for the clean up and analysis of PCDD/F, PCBs, PBDE and PCN in one clean up run will be presented. The standardized workflow and default methods used in this presentation, are ideally suited for high through put labs that want to optimizes their workflow, labs that want to switch from a manual to an automated workflow and of course for labs with no or low experience in the clean up of POPs.
1 Introduction:
In Japan, methods for measuring polychlorinated biphenyls (PCBs) are included in the Notification of the Ministry of Health and Welfare and the Manual for Determination of PCBs in Emissions. These methods suggest the use of sulfuric acid treatment, silica gel chromatography, or multilayer silica gel column chromatography for cleanup. Nevertheless, these methods have certain shortcomings, such as high processing time, high solvent consumption, complicated operation, and the requirement for skilled personnel to obtain stable and high recovery rates. Therefore, to achieve an accurate and reproducible PCBs analysis, the automated pretreatment system, GO-EHT (MIURA CO., LTD., Japan), originally developed for dioxin analysis, was applied as illustrated in Figure 1.

This system automates the cleanup of extracts containing dioxins and uses a pretreatment column consisting of a purification column and a concentration column. The purification column removes interfering substances coexisting in the extracts by heat treatment, and the concentration column adsorbs dioxins. The agent packed in the concentration column primarily comprises carbon, which adsorbs dioxins with coplanar structure, implying that so many PCBs isomers without coplanar structure are not adsorbed. In Japan, metal oxides, such as alumina, are used to adsorb the major isomers of the PCB products (Aroclor and Kanechlor). Therefore, the use of alumina instead of carbon as an agent in the concentration columns is one solution. However, a disadvantage of the use of alumina in the concentration columns is its inability to adsorb highly chlorinated tetraortho-PCBs, such as the IUPAC #209 which are not included in the PCB products. Therefore, an agent capable of adsorbing all 209 PCB isomers was needed. In this regard, we developed a transition metal oxide (TMO) as an alternative to alumina. In this study, we used TMO as the concentration column for GO-EHT and examined the elution conditions for PCBs. This system was also applied to river sediment-certified reference materials and actual samples.

2 Materials and Methods:
In this study, the PCB standard solutions were used the PCB Congener Mix 1~5 (Accustandard Inc.) which includes all 209 PCB isomers. In the cleanup process, TPCB-LCS-A500 was used as the cleanup spike and PCB-SS-A was used as the syringe spike (both from Wellington Laboratories Inc.). The automated pretreatment system GO-EHT (MIURA CO., LTD.) was used for the cleanup process. The pretreatment column consists of the purification column (packed with stacked silver nitrate silica gel at the top and sulfuric acid silica gel at the bottom) and the concentration column (packed with TMO) connected as shown in Figure 2. Measurement of PCBs was used high-resolution GC-MS (GC: GC7890 (Agilent Inc.), MS: JMS-800D Ultra FOCUS (JEOL Ltd.)). The GC inlet temperature was set at 280°C, and the temperature increase conditions were as follows: 120°C for 1 min → 20°C/min → 150°C → 1°C/min → 180°C → 3°C/min → 270°C → 10°C/min → 300°C for 6 min. Helium was used as the carrier gas and was maintained at a constant flow rate of 1.0 mL/min.

Figure 1: Automated sample preparation system (GO-EHT)

Figure 2: Pretreatment column
3. Results and Discussion:

3.1 The conditions for eluting PCBs from heated the purification column

An investigation was carried out under elution conditions for all 209 PCB isomers from the heated purification columns. The purification column was set to a heating temperature of 60℃, as used in dioxin analysis. Despite a significant increase in the hexane elution volume, monochlorinated biphenyls (MoCBs) were not eluted from the purification column, resulting in a very low recovery rate (0-10%). This was attributed to the strong adsorption of MoCBs on the heated silver nitrate silica gel. Therefore, to reduce this strong adsorption of MoCBs on silver nitrate silica gel, a small amount of polar solvent was added to the sample. After evaluation of various solvents and additives, the highest average recovery of all 209 PCB isomers was 102% at addition of 0.5 mL ethyl acetate. The hexane elution volume was 85 mL, which is the volume used in dioxin analysis. The IUPAC #3 recovery of the MoCBs significantly improved to 79%. These results are shown in Figure 3.

3.2 PCBs adsorption capacity of the concentration column packed with TMO

When cleanup was performed using only the purification column, the hexane eluate was concentrated after elution of the PCBs. However, this process can be complicated when a decompression concentrator is used, requiring time-consuming and careful work under mild conditions to prevent PCBs volatilization. Therefore, we developed TMO capable of adsorbing all 209 PCB isomers and used to the agent of the concentration column connected to the bottom of the purification column. Consequently, the PCBs eluted with hexane from the purification column were adsorbed onto the concentration column, and hexane was discharged as the waste liquid from the bottom of the concentration column. After eluting with hexane, PCBs were then eluted by reverse flow 3.2 mL toluene while the concentration column was heated to 90℃. This method concentrates PCBs in a small amount of toluene without the need for a decompression concentrator. The solution was further concentrated using a nitrogen stream, and a syringe spike was added to obtain a final liquid volume of 20 µL. This solution was used as the measurement solution. The results showed that the average recovery of all 209 PCB isomers was 90% satisfactory. These results are shown in Figure 4.

3.3. Results: of applying this system to certified reference materials of river sediments

This system was evaluated using the extracts of certified river sediment reference materials (JSAC 0431) obtained from the Japan Society for Analytical Chemistry (JSAC). As a result, good results were obtained for PCB homologues, with the deviation from the certified value divided by the standard deviation listed on the certificate within ±2. These results are shown in Figure 5.

3.4 Results: of applying this system to actual samples

The capabilities of this system were tested using the actual sample extracts. Five samples were used for each of soil, waste, exhaust gas, air, and food (fish oil). As a result, all measured values agreed with those of the conventional manual analysis within ±20% deviation. This system was shown to be applicable to a variety of PCBs analysis samples. These results are shown in Figure 6.

4 Conclusions:

This system can accurately quantify the concentration of all 209 PCB isomers in various samples by using TMO as the concentration column. This system also reduces the amount of solvent used compared to conventional methods, making PCBs analysis faster and simpler.

Figure 3: Recovery rate depending on the amount of ethyl acetate addition (1-3Cl)
Figure 4: Recovery rate of all 209 PCB isomers using TMO as the concentration column
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5 References:
1. Methods of Verification of Standards for Specially Controlled General Wastes and Specially Controlled Industrial Wastes, *Ministry of Health and Welfare Notification No. 192*

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**Figure 5:** Evaluation of the degree of deviation from the certified values of river sediment certified reference material (JSAC0431) in PCB homologues

**Figure 6:** Correlation of total PCBs concentration measured by this method and conventional method in actual samples
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Introduction: The continued interest in Persistent Organic Pollutants (POPs), such as polychlorinated dibenzo-p-dioxins (PCDDs), furans (PCDFs), biphenyls (PCBs), and organochlorine pesticides (OCPs) has led to a variety of systems for the cleanup of complex sample matrices. Manual techniques have been improved with both semi-automated and fully automated approaches.

Reasons for this include a) POPs sample processing is labor-intensive and prone to error. b) Compliance with regulatory procedures and accreditation requirements can result in a lengthy method validation effort. c) Strict quality assurance and quality control (QA/QC) requirements apply, and sample matrices can be very complex. d) Native background interferences can be orders of magnitude higher than analytes. Therefore, in most cases elaborate sample cleanup is needed.

A semi-automated system shown in Figure 1 was developed with a multi-pump and a sample processing unit. This unit can be used to process six samples in parallel. This system can be upgraded to a fully automated one with a valved sample processing unit that can be run unattended (Figure 2).

Both systems have some attributes in common: simple to run, fast (25-30 min), closed system with low chance of native cross-contamination, use of certified pre-packaged columns with no native background, low energy costs (multi-pump only), and choice of various column kits (size of acid silica column varies depending on amount of lipid in sample).

Materials and Methods:

Semi-automated

The semi-automated system uses a rotary workstation and a 6 channels parallel (multi-) pump to perform the entire sample cleanup in two stages (Figure1). It uses three pre-packaged columns: acidified silica of variable size (fat removal capacity 0.15-7 g), carbon and alumina. This is also the order in which the samples pass through the system which is different from the traditional silica-alumina-carbon order. The columns can be stacked on top of each other to form a column assembly.

Acidified silica (mixed with sulfuric acid) is used to oxidize lipids or other components of the sample (such as chlorophenols). Silver nitrate can be added to the column to remove any sulfur components present in the extract. Carbon columns can be used to further purify the extracts and to fractionate as it retains flat molecules such as all seventeen toxic PCDD/Fs and co-planary PCBs (# 77, 81, 126, 169) while letting other PCBs through. Finally, basic alumina is used to remove chlorobenzenes and other chlorinated components. The alumina column will retain mono- and di-ortho PCBs (# 105, 114, 118, 123, 156, 157, 167, 189).

The multi-pump has Start, Stop, and Halt commands on the touchscreen. It can accommodate six different solvents, although for standard operation as described here two solvents (hexane and toluene) are used. Each of the six positions on the workstation can be enabled from the multi-pump. A programmable flow rate (1.0 to 15.0 mL/min) and volume of solvent dispensed can be set from the multi-pump touchscreen. Columns back pressure for each of the six positions is also shown there. The system detects over pressure and halts pumps. The multi-pump also has a feature for a nitrogen push with both a pressure regulator and a pressure gauge below the touchscreen. The nitrogen can be used to push residual solvent during the sample loading/elution step onto the column assembly.

Solvents used are pesticide grade. Note that the cleanup uses hexane and toluene, no dichloromethane.

Stage 1 (all to waste): This is the stage where all solvent that is brought onto the column assembly goes to waste. Depending on the size of the acidic silica column, the silica-carbon-alumina assembly is conditioned with 20-60 mL of hexane. Samples described here can be extracts that already contain isotope dilution 13C PCDD/F standards or liquid samples that can be run on the system without prior extraction or treatment and are spiked at this point. Samples are then loaded onto the top of the acidic silica column using a syringe vial and at the same time eluted with the relevant amount of hexane (80-180 mL). The eluting hexane transfers PCDD/Fs and co-planary PCBs onto the carbon column and the mono- and di-ortho PCBs onto the alumina column. Residual hexane on top of the acidic silica in the syringe vial is pushed through the column assembly using nitrogen. After this step the syringe vials and silica columns are removed (discarded).

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Stage 2 (collect): Carbon and alumina columns are eluted individually in reverse direction with 40 mL toluene to collect the respective analytes. Two fractions are collected: Fraction 1 with PCDD/Fs and co-planary PCBs and Fraction 2 with the PCBs listed above. After sample collection, the extracts are transferred to a twelve-channel evaporator, which concentrates the 6 PCDD/Fs and 6 PCBs fractions. Direct-to-vial connections make the concentrated samples go directly into GC vials without further transfer, ready for GC/MS analysis. Samples are finally spiked with recovery standards and analyzed on an Agilent 7010B GC/MS/MS with 60m DB-5 type column.

Automated
The fully automated version of this system is shown in Figure 2. This system can be run unattended and has the valves shown. The program run is basically the same, but Stage 1 and Stage 2 are done with the valve configuration. The rotating workstation is no longer needed. The semi-automated system can be easily upgraded to the fully automated one by adding sample-processing module. Chemicals, columns, and volumes used are identical to the semi-automated system.

Figure 1: Semi-automated 6-channel PCDD/F and PCB cleanup system.
Figure 2: Fully automated 6-channel PCDD/F and PCB cleanup system.

The upgrade from semi- to fully automated can be done at low cost. In addition to PCDD/Fs and PCBs, other applications for both the systems include OCPs on a single Florisil column and Extractable Petroleum Hydrocarbon Fractionation, separating aliphatics from aromatics (PAHs) using a single neutral silica column (1).

Figure 3: $^{13}$C PCDD/Fs recoveries for oils on the semi-automated system.
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RESULTS: Figures 3 and 4 show average recoveries of the $^{13}$C PCDD/Fs and PCBs isotope dilution standards for the semi-automated and automated systems. All recoveries are 70% or higher. The matrices shown here are quite demanding and require significant lipid capacity. These samples were run with high-capacity acid silica columns which can handle up to 5 g of lipid (or oxidative) capacity.

DISCUSSION: The systems described here comprise a family of inexpensive cleanup equipment. The semi-automated system also has a vacuum version which uses a vacuum pump to do all the cleanup steps (2). That system does not use a multi-pump, but the sample processing rotary workstation is identical to the one described here, as are the columns and program used. The semi-automated system with the multi-pump and the fully automated system with elaborate valving are an extension of the vacuum system.

The recoveries shown in Figures 3 and 4 are comparable to those obtained with more expensive and modular cleanup equipment and demonstrate that with a simple automated system very good results can be obtained. In addition to PCDD/Fs and PCBs, the systems can also be used for OCPs and EPH cleanup.

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WE-AM-E1  Exploring the effectiveness of PCA, t-SNE, and UMAP for analyzing PCB fingerprints in NHANES 2003-2004 using silhouette scores

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Introduction: Serum levels of polychlorinated biphenyls (PCBs) reflect the body's burden by all routes of exposure, including ingestion, inhalation, and dermal absorption. The presence of PCBs in serum does not mean, by itself, that the chemical causes disease or an adverse effect as advancements in analytical chemistry allow for the measurement of extremely low levels of environmental chemicals. The assessment of exposure to PCBs can be done by comparing an individual to a general population with no known routes of exposure. In the United States, the baseline for the serum levels of PCBs is established through the National Health and Nutrition Examination Survey (NHANES) conducted by the Centers for Disease Control and Prevention (CDC)¹.². Beginning in the 1999-2000 and continuing into the 2003-2004 NHANES cycle, PCBs were measured in individual blood samples. Between 2005-2006 and 2015-2016 NHANES cycles (the latest study cycle reported for PCBs), blood samples from participants in the survey were pooled within each of the 40 demographic groups. Pooling of samples increased sample size and allowed better detectability of the targeted compounds. A line of evidence to assess for potential exposure is to examine the pattern of PCBs (PCB fingerprint) in the subjects' blood as compared to reference patterns, such as the weighted average NHANES pattern for the same demographic group as each of the subjects. If a unique exposure was involved, a distinct deviation between the "normal" PCB fingerprint found in the reference population would be evident. However, multivariate factors such as age, gender, ethnicity, and statistical population weightings will dictate the pattern of a "normal" PCB fingerprint. Multivariate dimensionality reduction techniques, such as principal component analysis (PCA), t-distributed stochastic neighbour embedding (t-SNE), and uniform manifold approximation and projection (UMAP) are environmental forensic tools in analyzing the complex and vast PCB fingerprint feature space derived from the NHANES dataset. By applying these dimensionality reduction techniques and limiting the feature space while maintaining key information, researchers can analyze subpopulations more effectively and identify correlations in a data matrix that contains many individual PCB fingerprints.

Method: As a data discovery and understanding tool of PCB fingerprints present in a non-exposed population, various methodologies of multivariate dimensionality reduction (PCA, t-SNE, UMAP) were applied to the 2003-2004 NHANES individual PCB serum data. To group similar PCB fingerprints into discrete subpopulations, we implement the unsupervised learning method using k-means clustering. Additionally, we use silhouette scores to quantify the grouping quality and evaluate the performance of the clustering algorithm for identifying unique fingerprinting groups.

Results: Through this data discovery pipeline, clear indications of an age-dependent PCB chemical fingerprint were apparent. Individuals 12-19 years of age demonstrated unique PCB profiles subjugated to lower chlorinated PCB congeners. Individuals in the largest age bracket (60+ years) demonstrated PCB profiles subjugated to more persistent PCB congeners reflective of a lifetime body burden.

Conclusion: Analysis of multiple multivariate dimensionality reduction techniques with unsupervised learning allowed us to identify correlations in the complex PCB fingerprint feature space. Our findings indicate clear age-dependent fluctuations in PCB chemical fingerprints, with unique profiles observed in the 12-19 age group and patterns reflective of a lifetime body burden in those above 60 years. The data pipeline associated with the discovery of these, "normal", PCB fingerprints provides another line of evidence in exposure assessment in concurrence with the upper margins from a non-exposed background population.

References:
1. Introduction:
The Environmental Specimen Bank (ESB) operated by the German Environment Agency (Umweltbundesamt, UBA) archives a unique collection of different sample types from across several ecosystem types in Germany. These samples comprise abiotic samples such as soil or suspended particles as well as biota samples such as bird eggs, fish tissue or tree leaves from different geographic regions. Samples cover terrestrial and aquatic (marine, limnic) ecosystems and different land uses such as sites located in near-natural ecosystem types, conurbations, forest and agrarian areas. In addition to these sites, the UBA operates air monitoring stations at several sites located in German background regions.

Polychlorinated Biphenyls (PCBs) are a well-known class of persistent organic pollutants which have been accumulated, transported and degraded within the ecosphere over several decades. In the past, PCBs were mainly used in form of technical mixtures, however, recently unintentionally formed PCBs became contaminants of emerging concern. In addition to its toxicological properties as well as different physical-chemical behavior triggered UBA's interest for assessing the PCB overall presence and distribution in Germany. Therefore, all 209 individual PCBs were analysed within two UBA projects. The first project investigated the 209 individual PCBs in a broad range of ESB samples dating – with few exceptions – from 2018 to 2021. The second project investigated the 209 PCBs in ambient air and deposition at two German background air monitoring sites (Waldhof, Schmücke) over a period of 12 months in 2018 and 2019. Among others, the following two questions were addressed: What is the present overall PCB contamination of environmental samples in Germany? And are typical (“technical”) and non-typical (“non-technical”) PCB patterns evident? A simplified approach was chosen to get insights to investigate aggregated levels and patterns on the basis of the degree of chlorination and comparison with the typical technical PCB mixtures. In the following, we present a first evaluation of the investigated data sets.

2. Materials and Methods:
Biota and abiotic samples (14 matrices = data sets) originate from the ESB long-term storage and monitoring and have been part of a research project covering environmental pollution by non-technical PCBs. Ambient air and deposition samples (4 data sets) originate from an UBA air monitoring project 2018/2019. Details regarding samples, sampling, storage and analytical procedure are described elsewhere. In brief, samples have been Soxhlet-extracted, cleaned by multistep column cleanup and analysed by GC-HRMS using a HT8-PCB column for all 209 PCBs, resulting in practically achievable 179 congener separations.

For the purpose of this publication, individual data for PCB congeners have been grouped and aggregated to sum congener groups for Mono-to DecaCB. Reference PCB congener distributions for technical mixtures were derived from Frame et al.5 and Schulz et al.6 and have been used to calculate chlorination degree totals and distributions (Figure 1). The relative contributions of congener group totals (PCB profiles, PCB patterns) were also calculated for the samples. Statistical analyses (descriptive analyses, correlation analyses, cluster analysis) were applied to evaluate the data set. For discussion, the samples have been assigned to aquatic, terrestrial and atmospheric ecosystems.
WE-AM-E2 Relative PCB Chlorination Patterns of the German Ecosphere – An Assessment to Predominant Technical Mixtures in Environmental Specimen Bank and Air Samples from the German Environment Agency

3. Results:
General data and summarised results for the PCB congener groups are presented in Table 1. The main results are given in Figure 2, where the PCB profiles are shown for all sample types (matrices). The profile of the technical PCB mixture in proximity to the PCB pattern of the respective environmental matrix types is indicated in this figure, too. The choice of the respective technical PCB mixtures presented in Figure 2 was based on the results of a correlation analysis which has been performed for all matrices against all technical PCB mixtures (Table 3). Furthermore, a cluster analysis was performed in order to group environmental samples and technical mixtures Figure 3).

Table 1: Sample descriptions and descriptive statistics

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Ecosystem</th>
<th>Sampling period (in brackets: single outliers)</th>
<th>Unit</th>
<th>Average</th>
<th>Median</th>
<th>RSD</th>
<th>Min-Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brenn muscle</td>
<td>aquatic (limnic)</td>
<td>8 2019-2021</td>
<td>µg/kg fw</td>
<td>224</td>
<td>126</td>
<td>118</td>
<td>11.2-847</td>
</tr>
<tr>
<td>Brenn liver</td>
<td>aquatic (limnic)</td>
<td>8 2019-2021</td>
<td>µg/kg fw</td>
<td>665</td>
<td>504</td>
<td>105</td>
<td>62.3-251</td>
</tr>
<tr>
<td>Eelpoint muscle</td>
<td>aquatic (nuritic)</td>
<td>3 2021</td>
<td>µg/kg fw</td>
<td>12.9</td>
<td>13.7</td>
<td>79</td>
<td>2.36-22.7</td>
</tr>
<tr>
<td>Zebra shell</td>
<td>aquatic (limnic)</td>
<td>4 2019-2020</td>
<td>µg/kg fw</td>
<td>13.9</td>
<td>13.5</td>
<td>94</td>
<td>0.26-28.5</td>
</tr>
<tr>
<td>Quegan muscle</td>
<td>aquatic (limnic)</td>
<td>6 2020-2020</td>
<td>µg/kg fw</td>
<td>27.0</td>
<td>25.6</td>
<td>69</td>
<td>7.61-52.2</td>
</tr>
<tr>
<td>Muscle</td>
<td>aquatic (limnic)</td>
<td>3 2019-2020</td>
<td>µg/kg fw</td>
<td>1.54</td>
<td>1.26</td>
<td>89</td>
<td>0.33-3.02</td>
</tr>
<tr>
<td>Suspended particulate matter</td>
<td>aquatic (limnic)</td>
<td>3 2015-2019</td>
<td>µg/kg dm</td>
<td>108</td>
<td>94.8</td>
<td>95</td>
<td>4.43-4302</td>
</tr>
<tr>
<td>Ferning soil</td>
<td>aquatic (nuritic)</td>
<td>8 2019-2021</td>
<td>µg/kg dm</td>
<td>522</td>
<td>398</td>
<td>51</td>
<td>345-827</td>
</tr>
<tr>
<td>Soil</td>
<td>terrestrial</td>
<td>9 (2014) 2018</td>
<td>µg/kg dm</td>
<td>17.3</td>
<td>18.7</td>
<td>73</td>
<td>1.27-39</td>
</tr>
<tr>
<td>Roe deer liver</td>
<td>terrestrial</td>
<td>5 2020</td>
<td>µg/kg fw</td>
<td>1.28</td>
<td>1.20</td>
<td>20</td>
<td>1.13-1.74</td>
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<tr>
<td>Earthworm</td>
<td>terrestrial</td>
<td>5 2013-2019</td>
<td>µg/kg dm</td>
<td>3.80</td>
<td>1.52</td>
<td>133</td>
<td>0.34-12.6</td>
</tr>
<tr>
<td>Spruce needles</td>
<td>terrestrial (atmospheric)</td>
<td>10 2018-2021</td>
<td>µg/kg fw</td>
<td>0.65</td>
<td>0.63</td>
<td>51</td>
<td>0.21-1.31</td>
</tr>
<tr>
<td>Poplar leaves</td>
<td>terrestrial (atmospheric)</td>
<td>2 2020</td>
<td>µg/kg fw</td>
<td>1.57</td>
<td>1.57</td>
<td>111</td>
<td>0.34-2.79</td>
</tr>
<tr>
<td>Beech leaves</td>
<td>terrestrial (atmospheric)</td>
<td>7 2019-2020</td>
<td>µg/kg fw</td>
<td>0.80</td>
<td>0.80</td>
<td>32</td>
<td>0.48-1.11</td>
</tr>
<tr>
<td>Ambient Air Schneeflock</td>
<td>atmospheric</td>
<td>12 2018-2019</td>
<td>µg/m³</td>
<td>43.5</td>
<td>48.3</td>
<td>34</td>
<td>13.1-66</td>
</tr>
<tr>
<td>Ambient Air Wildheide</td>
<td>atmospheric</td>
<td>12 2018-2019</td>
<td>µg/m³</td>
<td>48.5</td>
<td>48.2</td>
<td>21</td>
<td>36.4-67</td>
</tr>
<tr>
<td>Deposition Schneeflock</td>
<td>atmospheric</td>
<td>12 2018-2019</td>
<td>µg/(m².d)</td>
<td>1324</td>
<td>1517</td>
<td>69</td>
<td>1.36-3070</td>
</tr>
<tr>
<td>Deposition Wildhof</td>
<td>atmospheric</td>
<td>12 2018-2019</td>
<td>µg/(m².d)</td>
<td>2291</td>
<td>1851</td>
<td>99</td>
<td>126-7550</td>
</tr>
</tbody>
</table>

4. Discussion:
The PCB chlorination profile between the investigated samples from the ESB is statistically different to directly sampled atmospheric (air and deposition) samples. In ESB samples, HexaCBs contributed the most with further main contributions of Tetra, Penta and HeptaCBs and smaller contributions of PCBs with other chlorination degrees. Within the range of ESB matrices, the results indicate a general difference of PCB chlorination profiles between all examined aquatic sample types (here: fish, mussels/shell, suspended particulate matter) and air-related terrestrial sample types (here: tree leaves/needles) on one hand and the terrestrial samples (here: soil, roe deer liver, earthworm) on the other hand (Figure 2). Despite of the overall shares in the ESB samples, the standard variation of mean values (bars in Figure 2) is relatively low, irrespective of the sample’s concentration ranges, ecosystem types and sampling sites3. Thus, ESB samples overall show relatively uniform PCB profiles. In contrast, in atmospheric samples, PCB profiles comprise a wider range of main chlorination degrees: In ambient air samples, Di- to TetraCBs contributed the most, whereas in deposition samples a broad scattering of different chlorination degrees including significant shares of Hexa- and HeptaCBs as well as elevated standard deviations were observed. This result is probably influenced by a deposition contamination level close to or mostly below the method quantification limit.

Comparing ESB sample profiles to those of technical PCB mixtures, a strong similarity of all matrices to certain higher chlorinated mixtures, namely Clophen A60, one of the major German technical PCB mixtures, and Aroclor 1260 can be observed. This was corroborated by correlation and cluster analyses. The correlation analysis (Table 3) indicated for all ESB matrices correlation coefficients against Clophen A60 of 0.889 to 0.995 and for Aroclor 1260 of 0.779 to 0.971. For spruce needles and beech leaves, correlation coefficients were relatively low.
Furthermore, a notable correlation to the next lower chlorinated technical mixtures can be observed. The cluster analysis (Figure 3) yielded 4 clusters: One cluster (cluster C) consists of all terrestrial and aquatic samples together with the Clophen A60 / Aroclor 1260 technical mixtures but did not clearly distinguish the different sample matrices.

Air and deposition samples formed a second cluster together with all lower chlorinated technical PCBs (cluster A). The third and fourth clusters are formed by the remaining technical PCB mixtures of medium (cluster B) or very high chlorination degrees (cluster D), which seem not to contribute significantly to the observed PCB profiles of the samples.

Besides the dominance of Penta- to HeptaCBs, all aquatic samples are characterised by significant contributions of lower chlorinated PCBs, notably Tri-TetraCBs. In suspended particulate matter, also the shares of Mono- and DiCBs are somewhat more pronounced which is in contrast to all other ESB matrices. In the aquatic matrices, the higher chlorinated HeptaCBs contribute to a smaller degree to the total PCBs compared to the most similar technical PCB mixtures, with 11.9 % - 24.1 % HeptaCBs within the samples against 27.8 % HeptaCBs in Clophen A60 and 37.0 % HeptaCBs in Aroclor 1260. Compared against the most similar technical mixtures, this might possibly reflect a shift towards lower chlorination degrees, which are more volatile or water-soluble.

The herring gull egg samples have somewhat different profiles. The herring gull is part of the coastal ecosystem but has a feeding ecology based on nutrition from the terrestrial coastal catchment area before egg laying7. A contribution of terrestrial nutrition is also indicated by our data which show herring gull egg samples along with the terrestrial samples, i.e. with similarly small contribution of chlorination degrees below PentaCBs. The terrestrial samples related to atmospheric deposition (spruce needles, beech and poplar leaves) are bio-indicators of atmospheric pollutants such as PCBs8. In the present study, elevated shares of low to medium chlorinated PCBs (Tetra- to PentaCBs) was observed in these samples which is in contrast to the other terrestrial samples. Especially the occurrence of TriCBs in the spruce needle samples is noteworthy.
For air samples, the similarity to technical PCB mixtures was difficult to establish, considering the profiles presented in Figure 2 as well as correlation between matrices and the technical mixtures. Thus, the influence of single technical mixtures were difficult to assess. Furthermore, breakthrough of Mono and DiCBs during sampling in warm seasons could not be excluded, i.e. low chlorinated PCBs might even have contributed to a larger extent to the PCB profiles than shown by the data. Unintentionally produced PCBs of low chlorination degrees were described to be frequently present in elevated concentrations in such samples.

Compared to production volumes of technical mixtures, lower chlorinated PCBs (especially Tri-TetraCBs) are underrepresented in all ESB samples, particularly in terrestrial samples. E.g., more than 70 % of the German PCB production since their use in open systems ceased in 1974 consisted of PCBs with highest shares for TetraCBs (i.e. Clophen A30, A40). In the US, the respective Aroclor mixtures (i.e. Aroclor, 1221, 1232, 1016, 1242 and 1248) contributed for more than 60% to the total PCB production of technical mixtures for the US market (estimations calculated from publicly available data).

Table 3: Correlation coefficients (p < 0.05 highlighted in light green, p < 0.01 in middle green; correlation > 0.9 and most prominent tech. mixtures in bold)

Figure 3: Result of cluster analysis for reference technical PCB mixtures and sample matrices.
Further questions arise from the presented data. For example, there is a clear contribution of Decachlorobiphenyl to the PCB profiles of all matrices but air, deposition and needle/leaf samples. These shares are higher than in the technical PCB compositions, where DecaCB is not contributing at all, with the exception of the Aroclor 1260 mixture. This may point towards other sources of contribution to the total PCB pattern in the analysed samples, such as unintentional by-products.

5. Conclusions:
The present evaluation demonstrates the contribution of PCBs in the aquatic, terrestrial and atmospheric environments in Germany but also highlights the similarity of their profiles in a wide range of matrices within the different ecosystems investigated. However, without additional information on the PCB emissions from different PCB mixtures and their use (open/close systems), as well as possibly overlaying effects of e.g. metabolism and volatility, the discussion of the presented results calls for more data. Several questions remain unanswered, e.g. the effect of trophic levels of the sampled biota on the PCB pattern, the effect of short-term (year-to-year) variations or the effect of processes affecting concentrations and profiles on longer time scales. Thus, the data encourage a wider discussion about PCB sources and distribution processes within the total ecosphere for classic and novel PCB patterns, including non-technical mixtures.

6. Acknowledgments:
We thank the staff of the Waldhof and Schmücke monitoring sites for the support related to the air sampling. We thank the German Environment Agency, especially Andrea Minkos for supporting the air monitoring project.

The UBA is thanked for financial support (AZ Z 1.5 – 93 062/0004; FKZ 3717 522530).

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9. https://www.cdc.gov/niosh/docs/78-127/78127_7.html, as of 07.05.2023
Introduction: In this study polyurethane foam (PUF) disk passive air samplers (i.e., PUF-PAS) were applied to map emissions to air around two iron ore pelletizing facilities suspected of being important point sources of polychlorinated dibenzodioxins/furans (PCDD/Fs) to air.

Materials and Methods: PUF-PAS samplers were deployed for approximately 3 months around two facilities – one in Port-Cartier, Quebec and the other in Labrador City, Newfoundland/Labrador. At each facility, 15 samplers were deployed during the summer period (July-Sept, 2022) at varying distances around the perimeter of the facilities. Samples were prepared and analyzed by ALS, Burlington and deployed by Englobe Corporation, following the protocols of the Global Atmospheric Passive Sampling Network (Schuster et al., 2015). PCDD/Fs were analyzed by HRGC/HRMS using EPA methods 23 and TO-09A. Results were reported on a TEQ basis as fg/m³, based on the deployment time and a default PUF-PAS sampling rate of 4 m³/day and WHO TEF values.

Results: Concentrations of PCDD/Fs in air around the Port-Cartier facility indicated that it was a significant emissions source of PCDD/Fs to air, with concentrations ranging from about 4 fg/m³ TEQ to as high as 64.5 fg/m³ TEQ downwind of the facility; whereas results for the Labrador City facility (not shown) were relatively uniform across sampling sites with a mean value of 4 ± 2 fg/m³ TEQ, indicating less significance as a source.

Discussion and Conclusion: The results from the PUF-PAS mapping were used to estimate PCDD/F emissions to air for the Port-Cartier facility using a simple Gaussian dispersion model, \( E = C \frac{U D_y D_z}{D_y} \) (Cheng et al., 2011), where \( E \) is emission in fg TEQ/s, \( C \) is concentration in air fgTEQ/m³, \( U \) is windspeed (3.6 m/s), and \( D_y \) and \( D_z \) (m) are the height at which samplers were deployed (2m) and the horizontal fetch (distance from the sampling site), respectively. A ratio of \( D_z/D_y \) of 100:1 was used, which is representative of a neutral atmosphere (i.e., resulting in \( D_z = 200m \), which is within range for the study). Emissions were estimated to be approximately 3 gTEQ/year which is consistent the updated estimate for 2021 published under the National Pollution Release Inventory (NPRI) of 4.2 gTEQ/year in 2021. This study further illustrates the use of PUF-PAS as a simple tool for detecting and mapping chemical emissions to air (both gas and particle phase) associated with area sources or point sources such as industrial facilities.

Acknowledgments: Partial funding was provided by the Chemicals Management Plan. ALS for analytical support.

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Introduction:
PFAS are known as Poly and Per Fluorinated Alkyl Substances. It is a large group of estimated 4000 – 10,000 compounds from which only a few are well studied (type PFOA/PFOS). Poly Fluorinated compounds, which is the largest group, are not only from an analytical point difficult to determine but also they are subject to degradation phenomena which will lead in the long run to "new" degradation products being a Per Fluorinated type who are that stable that nature cannot degrade them anymore.
At present both legislators as well as consultants and laboratories are focusing only on a limited list of +/- 40 - 60 compounds and in the majority of cases only one technique (LC-MSMS) is used. There is a significant risk in underestimation of the actual PFAS presence in the environment as they are not covered from an analytical point of view.

Material and Methods:
By means of a multi-technique concept SGS has developed an approach which is based on following techniques:

Equipment:
- CIC method: AQF-210 A1-Science
- LC-MSMS: Sample Manager FTNH class H+ (Merk: Waters) Acquity Binary Solvent Manager Class I+ (Merk: Waters)/ Xevo TQ-XS MSMS (Merk: Waters)

Results:
Using the combination of techniques allowed us to reveal presence of poly-precursors compounds in environmental matrices as well as in AFFF/3F samples. The indicator parameters EOF/AOF where seen as a good indicator and as a predictor of non-targeted compounds (precursors). In a substantial number of cases (contaminations based on AFFF products), where target analysis showed "no PFAS", it was observed that elevate Organo Fluorine values as a predictor, and the consecutive TOP assay increased factors of a factor > 1000.

<table>
<thead>
<tr>
<th>EOF value, µg/kg</th>
<th>Target analysis, µg/kg</th>
<th>TOP assay &amp; post analysis, µg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>2500</td>
<td>&lt; 25</td>
<td>2300</td>
</tr>
</tbody>
</table>

Potential Human Risk
No indication for Human Risk

The above shows possible underestimation of PFAS presence if data are only based on Target analysis

Discussion and Conclusions:
By means of a combination of relatively new and existing analytical techniques (CIC – LC-MSMS) allowing to have a better/more complete picture on the presence for the majority of the estimated 4000 – 10,000 PFAS compounds and not only on a nowadays identified +/- 40 – 60 compounds. This means that both actual presence of known compounds style PFOA/PFOS are being identified and quantified as well as future deploying contaminations (due to degradation of actual long and unknown Poly Fluorinated Alkyl Substances). This will lead to close the gap between analytics & liability as analytical data are used for legal purposes.
WE-AM-E5  GC-HRMS-based suspect and non-target screening of organic micropollutants in groundwater in South Korea: Distribution characteristics and source identification using chemical indicators

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Introduction: Groundwater is a vital source of drinking water, making up 97% of the world's freshwater resources [1], but it is prone to contamination by organic micropollutants from various sources, including agricultural and industrial practices, landfill leachate, animal waste runoff, wet deposition, etc. Traditional analytical methods can only identify a limited number of target pollutants, leading to the omission of important potential chemical indicators not pre-selected for analysis. Therefore, a comprehensive and sensitive analytical approach that can screen for both target and non-target pollutants is necessary. GC-HRMS has emerged as a powerful tool for identifying a wide range of known and unknown organic pollutants via suspect and non-target screening (SNTS). GC-HRMS-based SNTS can potentially reduce the time and cost involved in acquiring reference standard materials for compounds that might not be detected in the analyzed samples. However, large amounts of data often generated from GC-HRMS-based SNTS require advanced computational methods to extract meaningful information. In this study, we aimed to explore the distribution patterns of pollutants in groundwater samples collected from various historically contaminated sites in South Korea. To achieve this, we employed a combination of suspect and non-target screening methods using GC-QTOF/MS. Additionally, we used robust multivariate statistical techniques to identify potential source-relevant pollution chemical indicators.

Materials and Methods: Groundwater samples (total n=78) were collected from various sites, including historical waste landfill (n=22), agricultural (n=15), industrial (n=12), and oil-contaminated (n=29) areas. Each sample, consisting of 500 mL, was subjected to solid phase extraction using 6cc HLB cartridges based on a validated method previously developed by our research group [2]. Subsequently, the extracted samples were analyzed using GC-QTOF/MS. To identify contamination patterns in the groundwater samples, a combination of an in-house personalized compound database and library (PCDL), containing retention times/mass spectra for 419 compounds, and the NIST 17.L commercial compound library, which contains nearly a million spectra for over 267,000 compounds, was utilized for data processing.

Results: A total of 251 compounds were identified by both suspect and non-target screening based on level 1 and 2 identification confidence, respectively, according to the criteria outlined by Schymanski et al. [3], and the identified compounds covered a wide range of pollutant groups, including parent polycyclic aromatic hydrocarbons (PAHs), alkylated PAHs, n-alkanes, phenols, plasticizers, synthetic musks, volatile methyl siloxanes, organophosphate flame retardants, benzo[a]chrysene ultraviolet stabilizers, pesticides, heterocyclic PAHs, hopanes/diamondoids, sesquiterpanes, pharmaceuticals, personal care products, transformation products, and others. To identify relevant chemical indicators for each contamination source, a combination of bootfs algorithm and PLS-DA model was utilized, and a total of 55 chemicals were flagged as important indicators. Detailed results and discussions will be presented at the conference.

Conclusions: The predictive ability (Q²) of our multivariate statistical model was 89.1%, and the results indicated that the contamination sources in the groundwater were mainly from anthropogenic activities. Our study contributes to a better understanding of the extent of organic micropollutant contamination in groundwater in South Korea and signals the need for the implementation of efficient management strategies to control groundwater pollution.

Acknowledgement:
This work was supported by a grant from Korean Environment Industry & Technology Institute (KEITI) through “Development of environmental forensics based on high resolution mass spectrometer to trace sources of subsurface contaminant Project” funded by Korean Ministry of Environment (1485017894) and Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2021R1A6A1A03039572).

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Introduction: Polycyclic aromatic hydrocarbons (PAHs) are widespread ecological hazards in the environment. Pollution sources have been identified by several methods based on concentrations and compositions of PAHs (Crockett and White, 2003; Katsoyiannis et al., 2014). In recent studies, compound-specific carbon stable isotope ratios have been proposed as a useful technique because isotopic indicators are more conserved than molecular indicators in the environment (O’Malley et al., 1994). In this study, we applied and compared both conventional molecular composition analysis and modern compound-specific isotope analysis (CSIA) techniques to identify sources of PAHs in sediments. The contributions of each PAH source were quantitatively evaluated using the CSIA results with the Bayesian isotope mixing model.

Materials and Methods: Fifteen traditional and 11 emerging PAHs were determined in sediments from 21 sources and 26 bay sites in Ulsan Bay, which is one of the most industrialized areas in South Korea. PAHs analysis was carried out by gas chromatography-mass selective detector after silica gel column clean-up. CSIA was conducted with an additional thin-layer chromatography clean-up, and significant CSIA results were obtained for four compounds (phenanthrene (Phe), fluoranthene, pyrene (Py), and benzo[a]anthracene). For comparison, source identification techniques based on molecular composition included diagnostic ratios and positive matrix factorization models. The contribution of pollution sources was quantitatively evaluated using a Bayesian isotope mixing model that utilized the CSIA results.

Results: The concentrations of traditional PAHs in sediments from potential source sites varied widely, ranging from 290 to 14,000 ng g⁻¹ organic carbon (OC). Emerging PAHs were also found with widely varying concentrations (21–2000 ng g⁻¹ OC), and their distribution showed a strong correlation with the distribution of traditional PAHs (r = 0.8, p < 0.05). Compositional analysis of PAHs revealed that high molecular weight PAHs containing 4–6 aromatic rings were three times more abundant than low molecular weight PAHs containing 2–3 aromatic rings. The source identification results of the diagnostic ratios and positive matrix factorization model were inconsistent in adjacent source areas. In urban sediments, the lighter δ¹³C_Phe was observed (mean: –25.1‰), while in petroleum industry areas, the relatively heavier δ¹³C_Py was found (mean: –23.4‰). The Bayesian isotope mixing model indicated that the predominant source of PAHs in Ulsan Bay sediments was the petroleum industry (45%), followed by the non-ferrous metals industry (30%), automobile industry (18%), and urban areas (6.3%).

Discussion and Conclusion: Environmental management is crucially dependent on the ability to identify pollution sources within an area. However, methods based on molecular compositions may not be accurate or conclusive in regions where significant compositional changes occur in the environment. Within Ulsan Bay, concentrations and compositions of PAHs change rapidly, while stable isotope ratios of these pollutants are relatively conserved during transport through the environment. Our study demonstrates that these conservative isotopic methods enable the identification of pollution sources in a complex and dynamic coastal environment.

Acknowledgment: This research was supported by the "Development of Source Identification and Apportionment Methods for Toxic Substances in Marine Environments (20220534)" program of the Korea Institute of Marine Science & Technology Promotion (KIMST) funded by the Ministry of Oceans and Fisheries.

References:
Introduction: Around the globe, the legacy use of per- and polyfluorinated substances (PFAS) in aqueous film-forming foams (AFFFs) has resulted in many PFAS-contaminated source zones continuing to release PFAS to surrounding environmental receptors.\(^1\)\(^2\) Many polyfluorinated substances can transform into persistent perfluoralkyl acids (so-called PFAA precursors) and/or semi-stable perfluorinated intermediates in the environment.\(^3\) The presence of such precursors in source-zone soils can result in the release of PFAAs in environmental receptors on-site/downgradient of the source zone due to PFAS mobilization/transport coupled with slow transformation.\(^4\)\(^5\) The aim of this study was to investigate the transformation behaviour in aerobic soils of three shorter carbon (C)-chain (C ≤ 6) suspected AFFF-related perfluoroalkyl acids (PFAA) precursors having different hydrophilic functional groups.

Material and Methods: Soil microcosms were set up using two loamy soils including an Entisol (pH 6.6, organic carbon content – OC 3.2 %) and a Spodosol (pH 4.8, OC 1.5%) under live and sterilised conditions. The studied zwitterionic PFAS included perfluorohexane sulfonamidoalkyl ammonium (TAmPr-FHxSA, electrochemical fluorination (ECF)-derived), 5:3 fluorotelomer betaine (5:3 Fb, fluorotelomer (FT)-based), and 6:2 fluorotelomer sulfonamidoalkyl betaine (6:2 FTSA-PrB, FT-based). Both targeted and suspected analyses were conducted for quantifying and identifying transformation products and/or intermediates.

Results: The transformation half-lives ranged from several months to years depending on the functional head groups and the soil type. The betaine head group showed the highest stability, followed by the sulfonamide betaine group and the sulfonamide quaternary ammonium group. Transformation pathways and intermediates/terminal products of the same precursor were affected by soil properties.

Discussion and Conclusion: Although there could be a potential for both biotic and abiotic transformation processes to occur simultaneously, our data suggest that biotic transformation processes are major drivers for the transformation of these zwitterionic PFAS precursor compounds in aerobic soils. In addition, the shortening of perfluorinated C-chain or partial defluorination of precursors in this study can be linked to the presence of short-chain PFAS at AFFF-contaminated sites for decades, even after AFFF use has been discontinued and despite being highly mobile in soil. Given the abundance of related zwitterionic-type compounds at contaminated sites, this highlights the need for more attention on those compounds in source-zone remediation/management activities.

References
Per- and Polyfluoroalkyl Substances (PFAS) in Sewage Sludge and Wastewater-based Fertilizers

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Introduction: Per- and polyfluoroalkyl substances (PFAS) are chemicals which were developed to improve humanity’s quality of life. Due to their high chemical stability and resistance to degradation by heat or acids, PFAS were used in a variety of consumer products. The continuous use of PFAS in household products and the discharge of PFAS from industrial plants into the sewer system resulted in the contamination of effluents and sewage sludge from wastewater treatment plants (WWTPs) (Roesch et al. 2022). Since sewage sludge is often used as fertilizer, its application on agricultural soils has been observed as a significant entry path for PFAS into the environment, specifically in our food chain. In Germany the sewage sludge/biosolid application on agricultural land was banned with the amendment of the German Sewage Sludge Ordinance and by 2029 sewage sludge application will be totally prohibited. However, phosphorus (P) from sewage sludge should still be recycled in WWTPs of cities with a population larger than 50,000 residents. To produce high-quality P-fertilizers for a circular economy, PFAS and other pollutants (e.g. pesticides and pharmaceuticals) must be separated from sewage sludge. Due to the strong diversity of industrial PFAS usage it is not clear if a safe application of novel recycled P-fertilizers from WWTPs can be guaranteed. Therefore, we analyzed various sewage sludges and wastewater-based fertilizers.

Materials and Methods: Sewage sludge (SL) samples from various WWTPs in Germany and Switzerland, six sewage sludge ashes (SSA) from Germany, six thermally treated SL and SSA samples with different additives (temperatures: 700–1050 °C), two pyrolyzed SL samples (temperature: 400 °C) and two struvite samples from Germany and Canada were analyzed. The goal was to quantify PFAS in sewage sludges and wastewater-based P-fertilizers with the sum parameter extractable organic fluorine (EOF) by combustion ion chromatography (CIC). The results were compared with data from classical LC-MS/MS target analysis as well as selected samples by HR-MS suspect screening.

Results: The EOF values of the SLs mainly range between 154 and 538 µg/kg except for one SL which showed an elevated EOF value of 7209 µg/kg due to high organofluorine contamination. For the SSA samples the EOF values were lower and values between LOQ (approx. 60 µg/kg) and 121 µg/kg could be detected. For the pyrolyzed SLs no EOF values above the LOQ were detected. Moreover, the two wastewater-based struvite fertilizers contain 96 and 112 µg/kg EOF, respectively. In contrast to the EOF values, the sum of PFAS target values were relatively low for all SLs. Additional applied PFAS HR-MS suspect screening aimed to tentatively identify PFAS that could contribute to the hitherto unknown part of the EOF value. The majority of the detected fluorinated compounds are legacy PFAS such as short- and long-chain perfluorocarboxylic acids (PFCA), perfluorosulfonic acids (PFSA), polyfluoroalkyl phosphate esters (PAPs) and perfluorophosphonic acids (PFPA). Moreover, fluorinated pesticides, pharmaceutical as well as aromatic compounds were also identified, which are all included in the EOF parameter.

Discussion and Conclusion: Our research revealed that the current PFAS limit of 100 µg/kg for the sum of PFOS + PFOA in the German Fertilizer Ordinance is no longer up to date. Since the number of known PFAS already exceeds 10,000, the ordinance limit should be updated accordingly. Recent regulations and restrictions on using long-chain PFAS (≥C8) have resulted in a significant shift in the industry towards (ultra-)short-chain alternatives, and other, partly unknown, emerging PFAS. Ultimately, also fluorinated pesticides and pharmaceuticals, which end up as ultrashort PFAS in the WWTPs, have to be considered as possible pollutants in fertilizers from wastewater, too.

Acknowledgments:
The authors thank the German Federal Ministry of Economic Affairs and Climate Action (BMWK; ZIM program 16KN076702 "PerFluSan-PFTSan" and 16KN076724 "MIDRAPA") for funding.

References:
Introduction: PFAS are a class of contaminants that have been in the spotlight since the awareness of their toxicity. Their presence was reported in different kinds of matrices such as air, wastewater, surface and drinking water, sediments, fish, and even human blood.[1] With this widespread, global distribution in all environmental and human compartments, in combination with the knowledge of their toxicity, the production of alternative PFAS increased. In this context, monitoring WWTP influent samples can be used as proxy to assess how the population is exposed to PFAS and can be used for regulatory purposes.[2] Besides that, applying nontarget and suspect screening to WWTP will give more information regarding emerging PFAS that are being produced. Knowledge of the structure and identity of novel PFAS chemicals is also required for the accurate quantification and toxicity assessment, for which native and labeled standards are crucial. These are often not available, leading to significant knowledge gaps on fate and effects of these chemicals. This work presents a suspect and non-target screening approach to identify emerging PFAS in WWTP samples from six locations in the Netherlands using FluoroMatch[3] software for in-depth data analysis and identification. Further, with this study, we aim to synthesize the native/labeled standards for the compounds identified in the screening.

Materials and Methods: Six WWTPs in the Netherlands were selected, and the samples were extracted through solid-phase extraction with HLB cartridge, followed by dispersive solid-phase extraction. For the target analysis, LC-MS/MS was performed. The suspect and nontarget screening was performed using a Quadrupole time-of-flight (QTOF) mass spectrometer. Feature detection (e.g., peak picking, deconvolution, and alignment) was performed using mzMine. The obtained feature list was then fed to FluoroMatch for further feature annotation and (tentative) identification.

Results: As expected, the target analysis revealed that the locations most contaminated by PFAS were the two directly influenced by industrial production of PFAS (Dordrecht and Bath). To increase both sensitivity and identification rate, samples were first analysed in full scan to record all MS1 information. For those features which showed a potential match with available libraries based on MS1 data, a preferred list was prepared to collect high quality MS2 data. The samples were then reanalysed using this method and the obtained data was further processed with FluoroMatch.

Discussion and Conclusion: From the six Dutch WWTPs, the target analysis revealed two locations that process industrial wastewater showed to have the highest total PFAS concentrations (880 and 1128 ng/L, sum of the concentration of 36 PFAS determined using isotopically labeled) and it was possible to detect legacy PFAS (PFOA, PFBA, and PFBS) along with emerging PFAS chemicals (6:2 FTS, MeFBSAA, and Gen-X). The LC parameters used for the suspect and nontarget screening showed a good separation and the optimization of the parameters for the MS/MS results were crucial for a good fragmentation of the contaminants. FluoroMatch software was an effective tool to annotate and identify new PFAS classes, of which examples will be shown. This research demonstrates the need to monitor the production and release of new alternatives, as well as the need for the production of native and labelled standards to evaluate the concentration of emerging PFAS in the environment as well as their toxicological properties.

Acknowledgments: We thank the Eureka-Eurostars programme, the EU Framework Programmes H2020-MSCA-ITN-2020 under the Marie Skłodowska-Curie grant agreement REVAMP project No. 956374.

References:
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1. Introduction:
The work focuses on the identification of short-chain, polar per- and polyfluoroalkyl substances (PFAS) in effluent samples using reference materials synthesized beforehand as a part of the European 'REVAMP' PhD project. Short-chain replacements to perfluorooctane sulfonyl derivatives including N-alkyl perfluoralkane sulfonamides, N-alkyl perfluoralkanesulfonamidoacetic acids, N-alkyl perfluorooctane sulfonamidoethanols and N-alkyl perfluorooctanesulfonamidomethylacrylates were selected. This kind of compounds are more and more used to replace PFAS that are known for their impact on the environment and human health like PFOA (perfluorooctanoic acid), PFOS (perfluorooctanesulfonic acid) and their long chain derivatives1,2. PFAS identified or suspected in AFFFs (aqueous film-forming foams) such as perfluorooctanesulfonamidoamino amines or perfluorooctanesulfonamidomethyl quaternary ammonium salts were also included in this study3,4. Regarding the nature of the compounds, effluent samples were targeted in the first place. Samples were extracted by a SPE method and then PFAS were analysed either by liquid or gas chromatography- mass spectrometry for identification and quantification.

2. Materials and Methods:
Reference standards used in this work were all synthesized at Chiron (Trondheim, Norway), namely N-methyl and N-ethyl perfluorooctanesulfonamidomethylacrylate (C4 and C6, MeFBSAA, EtFBSAA, MeFBSAmS, EtFBSAmS), N-methyl and N-ethyl perfluorooctanesulfonamidomethylacrylate (C4 and C6, MeFBSAA, EtFBSAA, MeFBSAmS, EtFBSAmS), N-methyl and N-ethyl perfluorooctanesulfonamidomethylacrylate (C4 and C6, MeFBSAA, EtFBSAA, MeFBSAmS, EtFBSAmS), N-methyl and N-ethyl perfluorooctanesulfonamidomethylacrylate (C4 and C6, MeFBSAA, EtFBSAA, MeFBSAmS, EtFBSAmS), N-methyl and N-ethyl perfluorooctanesulfonamidomethylacrylate (C4 and C6, MeFBSAA, EtFBSAA, MeFBSAmS, EtFBSAmS) and their deuterated analogues, 2:2 fluorotelomer sulfonate (6:2 FTS), N-dimethyl ammonium propyl perfluorooctane sulfonamide (AmPr-FBSA, AmPr-FHxSA, AmPr-FOSA), perfluorooctanesulfonamidomethyl quaternary ammonium salt (PFBSAmS, PFHxSAmS, PFOSAmS), N-perfluorobutane sulfonamidoethanol (FBSE), 1-propanesulfonic acid, 3-[[3-[dimethylamino]propyl][[(tridecafluorohexyl)sulfonyl]amino], 6:2 fluorotelomer sulfonyl propanoic acid and its thioether analogue (6:2 FTSO2PrA and 6:2 FTThPrA), N-sulfopropyl perfluorohexanesulfonamide (6-SP-FHxSA). They were received either as a solution in methanol (50 mg/mL) or as neat crystals (between 1 and 2 mg). In the latter case, solutions were prepared in methanol (between 1 mg/mL and 2 mg/mL). Methanol for Ultra LC-MS was provided by Carl Roth (Karlsruhe, Germany), isoctane for gas chromatography, and ammonia solution 25% for HPLC were supplied by Merck (Darmstadt, Germany), and acetic acid glacial for ULC/MS by Biosolve Chimie (Dieuze, France). Ultrapure water was supplied by a Milli-Q system from Millipore (Watford, UK).

Ten effluent samples were collected in the Netherlands between 2022 and 2023. The list of the samples and their location are described below (Table 1). These samples were already analysed for other PFAS (PFOA, PFOS, 4: FTS, 6:2 FTS, 8:2 FTS, 10:2 FTS, Me/EtFOSAA, Me/EtFOSAA, PFBS, PFHxS, PFBA, FHXSA, FOSA, DONA, HFPO-DA, PFPeS, PFNS, PFHpS, 8:2 diPAP, FBSA, PFPeA, PFNA, PFPeA, PFHxA, PFHpA) but the screening was updated with the synthesized compounds. One of these samples was used for a spiking experiment. Samples were extracted with OASIS-WAX SPE cartridges (150 mg, 30 µm, Oasis®). The cartridges were conditioned with 4 mL 0.1% NH₄OH in methanol followed by 4 mL methanol, and 4 mL water. After loading of 25 mL for each sample containing 50 µL of internal standard, cartridges were washed with 4 mL 1% acetic acid then freeze dried for 2 hr. PFAS were eluted with 4 mL 0.1% NH₄OH in methanol. Solvent was evaporated before reconstitution in 25 mL Milli-Q water and 25 µL methanol or in 50 µL isoctane. PFASs were analysed either by a SCIEX 6500+ triple-quadrupole mass spectrometer with electrospray ionization (SCIEX, Amsterdam, The Netherlands) or with an Agilent 7000D Triple Quad GC-MS/MS (Agilent Technologies, Amsterdam, The Netherlands).
THU-AM-A4 Occurrence of Short-Chain, Polar PFAS in Effluent Samples

Table 1: Sewage treatment plant samples from the Netherlands analyzed.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Location</th>
<th>Matrix</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number 0</td>
<td>Weert</td>
<td>Effluent</td>
</tr>
<tr>
<td>Number 1</td>
<td>Leiden</td>
<td>Effluent</td>
</tr>
<tr>
<td>Number 2</td>
<td>Kralingseveer</td>
<td>Effluent</td>
</tr>
<tr>
<td>Number 3</td>
<td>Nieuwerkerk a/d Ijssel</td>
<td>Effluent</td>
</tr>
<tr>
<td>Number 4</td>
<td>Aarle-Rixtel</td>
<td>Effluent</td>
</tr>
<tr>
<td>Number 5</td>
<td>Dordrecht</td>
<td>Effluent</td>
</tr>
<tr>
<td>Number 6</td>
<td>Ooijen</td>
<td>Effluent</td>
</tr>
<tr>
<td>Number 7</td>
<td>Asten</td>
<td>Effluent</td>
</tr>
<tr>
<td>Number 8</td>
<td>Bath</td>
<td>Effluent</td>
</tr>
<tr>
<td>Number 9</td>
<td>Tilburg</td>
<td>Effluent</td>
</tr>
</tbody>
</table>

3. Results:
So far, four of the target compounds were analyzed as a first test, namely MeFBSA, MeFBSAA, MeFHxSA, and MeFHxSAA. Three of them were identified in the samples. MeFBSAA was found in all of them (Table 2). Concentrations of MeFHxSA were lower than the detection limit of 0.4 ng/mL.

Table 2: Identification of some targeted compounds in effluent samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>MeFBSA</th>
<th>MeFBSAA</th>
<th>MeFHxSA</th>
<th>MeFHxSAA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number 0 (spiked)</td>
<td>✓</td>
<td></td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>Number 1</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Number 2</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Number 3</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number 4</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number 5</td>
<td>✓</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Number 6</td>
<td>✓</td>
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<td>✓</td>
</tr>
<tr>
<td>Number 7</td>
<td>✓</td>
<td></td>
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<td>Number 8</td>
<td>✓</td>
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<td>✓</td>
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<tr>
<td>Number 9</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td>✓</td>
</tr>
</tbody>
</table>

Concentrations of the detected PFAS vary in the different samples from less than 1 ng/mL to more than 60 ng/mL. As an example the concentrations of some compounds for two samples are given below (Figure 1 and 2). In some cases, detected concentrations are higher than the well-known PFAS such as PFOS or PFOA. For example, in sample 4, the concentrations of MeFBSAA and MeFBSA are higher than those of PFOS (2.2 and 2 ng/mL for the short-chain analogues and 1.7 ng/mL for PFOS). In sample 9, the concentration of MeFBSAA is almost two times the concentration of PFOA (Figure 1 and 2).
THU-AM-A4  Occurrence of Short-Chain, Polar PFAS in Effluent Samples

Figure 1: Concentration of targeted PFAS in sample 4 (ng/mL) compared with other PFAS previously measured.

Figure 2: Concentration of targeted PFAS in sample 9 (ng/mL) compared with other PFAS previously measured.
THU-AM-A4 Occurrence of Short-Chain, Polar PFAS in Effluent Samples

4. Discussion:
MeFBSAA was identified in every sample. In some cases, concentrations of these short-chain compounds are higher than their long-chain analogues previously used by the industry. Samples containing these short chain N-alkyl perfluorooctane sulfonamide derivatives also contain short-chain analogues of PFOS. Different studies have established links between these compounds. Indeed, N-alkyl perfluorooctane(butane) sulfonamidoacetic acid can be degraded to N-alkyl perfluorooctane(butane) sulfonamide before leading to PFOS or PFBS5,6,7.

5. Conclusions:
MeFBSAA, MeFBSA and MeFHxSAA were identified and quantified in different effluent samples. Concentrations were in some cases higher than those of the well-known long-chain PFAS such as PFOA or PFOS that are frequently analysed. Other samples will be analyzed by including the other reference materials as listed in Materials and Methods.

6. Acknowledgments:
We thank the EU Framework Programme H2020-MSCA-ITN-2020 under the Marie Skłodowska-Curie grant agreement No 956374 for funding this study.

7. References:
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Introduction:
Per- and polyfluoroalkyl substances (PFAS) are currently the subject of much discussion in Europe due to the proposed restriction of the entire group of PFAS unless a specific use is proven essential for society [1]. Even though emissions of long-chain perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkane sulfonic acids (PFSAs) as well as the replacement substance hexafluoropropylene oxide dimer acid (HFPO-DA) have been reduced [2, 3], their high persistence has led to irreversible environmental exposure and the compounds are predicted to remain in the environment for hundreds of years. As the ocean is considered a major sink for PFAS, understanding the land-ocean transfer of PFAS in coastal regions is important to assess the compounds’ fate and impact in the upcoming decades.

Prior studies have investigated PFAS in North Sea coastal regions [4, 5, 6] and at selected locations along the Atlantic coast [7]. Riverine outflows have been identified as a main source of PFAS in coastal regions [8]. Because most studies focus on surface water samples, field data on the vertical distribution of PFAS in the water column of coastal and open ocean regions is still lacking. However, this data is needed to reduce uncertainties in global PFAS mass balances and assess the role of the oceans as a sink of PFAS. Using conventional compound-specific analytical methods, only dozens out of several thousand PFAS on the global market are determined. While non-target or suspect screening has been frequently applied to contaminated sites and products such as aqueous film forming foams (AFFFs) in the last years [9], such studies unravelling the unknown share of PFAS in coastal and open ocean regions are still rare.

The objectives of this study were i) to use target analysis to investigate the occurrence and distribution of 37 PFAS, especially ether-based replacement substances and other emerging PFAS, along the Western European coastline, ii) to compare PFAS composition patterns related to the outflows from major river systems, iii) to provide knowledge on the vertical distribution of PFAS in coastal regions, iv) to perform a PFAS suspect screening in seawater sample extracts from today, 2007, 2011, 2014 and 2017.

Materials and Methods:
Seawater samples (1-L each) were collected during expedition AL534/2 of the research vessel Alkor along the western European coastline from Malaga (Spain) to Kiel (Germany) in March 2020. 36 surface water samples were taken using the ship’s seawater intake system (Figure 1a). Additionally, seawater from three to six depths was collected using the CTD/rosette sampler at five stations. The samples were stored at 4 °C. Back at Helmholtz-Zentrum Hereon, they were extracted based on a protocol optimized for seawater samples [6]. Briefly, the filtered 1-L water samples were spiked with isotope-labelled internal standards (50 µL, 60 pg/µL) and loaded onto preconditioned solid phase extraction cartridges (Waters Oasis WAX; 6cc, 500 mg, 30 µm). After a washing step, the target compounds were eluted using methanol and 0.1 % ammonium hydroxide in methanol. The eluates were reduced to 150 µL under nitrogen and [13C8]-PFOA was added as injection standard (10 µL, 100 pg/µL). The sample extracts were analyzed for 37 PFAS using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). The scope of target analytes included 11 PFCAs (C4 to C14), five PFSAs (C4, C6, C7, C8, C10), the cyclic PFSA perfluoro-4-ethylcyclohexanesulfonate (PFECHS), seven per- and polyfluoroether carboxylic acids (PFECAs) (HFPO-DA, hexafluoropropylene oxide-trimer and tetramer acid (HFPO-TeA, HFPO-TrA, HFPO-TeA); 4,8-dioxa-3H-perfluorononanoic acid (DONA), perfluoro-4-oxapentanoic acid (PF4OPeA), perfluoro-5-oxahexanoic acid (PF5OHxA), perfluoro-3,6-dioxaheptanoic acid (3,6-OPFHpA)), three per- and polyfluoroether sulfonic acids (PFESAs) (perfluoro(2-ethoxyethane)sulfonic acid (PFEEESA), 6.2 and 8.2 Cl-PFESA), two perfluoroalkyl phosphinic acids (PFPIAs) (6.6 PPfIA, 6.8 PPfIA), and seven precursors to PFCAs and PFSAs (FOSA, N-EtFOSA, N-EtFOSE and N-EtFOSAA; 4.2 FTSA, 6.2 FTEA, 8.2 FTSAs).

For the retrospective screening, a selected number of samples from this campaign and stored samples extracts from 2007/8, 2011, 2014 and 2017 were analyzed by liquid chromatography coupled to a quadrupole time-of-flight mass spectrometer (LC-QToF). The componentized raw data was screened for 4777 PFAS using the suspect list “S89” from the NORMAN suspect list exchange (SLE), created by merging other SLE lists and searching for additional content in the NORMAN SusDat database [10].
Results and Discussion:
The sum of PFAS ranged from 290 pg/L to 17000 pg/L in the German Bight and English Channel (mean 5500 pg/L), whereas levels were approximately 10 times lower along the Atlantic coastline, ranging from 150 pg/L to 850 pg/L (mean 270 pg/L) (Figure 1b). Of the ether-based replacement PFAS, HFPO-DA was detected in 97 % of the surface water samples (Figure 1c). With a mean concentration of 23 pg/L, HFPO-DA had a smaller share of the total PFAS sum (3 ± 2 %) compared to PFCAs such as PFBA (28 ± 11%) and PFHxA (14 ± 7%).

Compared to earlier measurements in the German Bight, concentrations of legacy PFCAs and PFSAs as well as HFPO-DA decreased significantly. As an example, HFPO-DA levels decreased from 1600 ± 300 pg/L in samples from 2017 [6] to 61 ± 10 pg/L in samples from 2020 [this study]. A possible explanation is that the emission permit of the fluoropolymer manufacturer in the Netherlands, which is assumed to be the major source for HFPO-DA in North Sea waters [6], was lowered from 2035 kg/year in 2017 to 148 kg/year in 2019 [3].

Of the other ether-based replacement PFAS, DONA was not detected, while its degradation product PF4OPeA was present in 40 % of the surface water samples, mainly in the German Bight. In addition, the emerging cyclic compound PFECHS was detected in 13 % of the surface water samples. Its occurrence correlated with comparatively high concentrations of PFOS. This can be due to the presence of PFECHS as an impurity in perfluorooctanesulfonyl fluoride(POSF)-based products, such as aqueous film forming foams (AFFFs) [12].

Figure 1: a) Locations of surface water samples (red circles). Yellow stars mark the CTD profiles discussed below. Distribution of b) sum of PFAS with a detection frequency >50%, c) HFPO-DA d) PFBA along the cruisetrack. Data was plotted using Ocean Data View [13].
Per- and Polyfluorinated Substances (PFAS): Occurrence and Exposure
L.Bervoets & J.Stubleski

Samples from stations chosen to target outflows from river systems with high anthropogenic influence showed the highest concentrations of the sum of PFAS. The PFAS composition pattern varied between the different river systems. The short-chain PFCA PFBA had the highest share in the outflow of the Rhine River (47 %) (Figure 2). This may be related to its use as intermediate for the production of pharmaceuticals and pesticides in chemical parks located at the Rhine River. The outflow of the Seine River was characterized by a comparatively high proportion of the precursor compound 6:2 FTSA (14 %) (Figure 2). This corresponds to the finding of a sharp increase of 6:2 FTSA in the lower Seine River in a previous study, likely reflecting industrial and/or urban inputs [14].

The vertical distribution of PFAS varied between the available profiles. At station 10, close to the outflow from the Seine River, the sum of PFAS decreased significantly from 2800 pg/L at 4 m depth to 700 pg/L at 10 m depth (Figure 3b). On the contrary, at station 8, in the English Channel (Figure 1a), the PFAS concentrations and pattern showed comparatively small variations at the six different sampling depths (330±56 pg/L, Figure 3a). The salinity profile obtained at station 8 showed a constant salinity of 35.29±0.02 PSU from the surface down to 70 m depth, indicating that the water was mixed well (Figure 3a). In contrast, at station 10 there was a top layer with low salinity of 20-30 psu down to 5 m depth and a constant higher salinity of 34.19±0.02 PSU at greater depths (Figure 3b). The lower salinity and higher PFAS concentrations in the sample taken in the top water layer at station 10 can be explained by outflow from the Seine River.

Preliminary results of the retrospective PFAS screening in surface water sample extracts from the German Bight indicate that the replacement compound HFPO-DA was present in the River Rhine delta in 2008 and in the German Bight in 2011 already.
Per- and Polyfluorinated Substances (PFAS): Occurrence and Exposure

L. Bervoets & J. Stubleski

THU-AM-A5 Legacy and Emerging Per- and Polyfluoroalkyl Substances (PFAS) along the Western European Coast – Riverine Outflows, Vertical Profiles and Retrospective Screening

At this time, an analytical standard for this compound had not been available and the discussion on potential adverse effects of replacement PFAS had not started yet. This shows the limitations of target analysis focusing on a predefined scope of well-known PFAS.

Conclusions:
Differences in PFAS concentrations and patterns observed in this study reflect the influence of riverine outflows and underline the role of rivers as major sources of PFAS in coastal regions. The observed differences in the distribution of PFAS along the vertical water column indicate that knowledge of vertical and lateral stratification along with ocean circulation is crucial for understanding the large-scale distribution and fate of PFAS in the ocean and reduce uncertainties in global PFAS mass balances.

The decrease of PFAS levels in the German Bight shows changes in pollution levels as a consequence of action taken by regulatory authorities and industry. However, the occurrence of the degradation product of DONA and the detection of HFPO-DA in samples from periods when fluorinated alternatives had not yet been in focus show the limitations of target analysis focusing on a predefined scope of well-known PFAS. Moreover, it underlines the importance of a grouping approach on a regulatory level.

6. Acknowledgments:
The authors gratefully thank the captain and the crew of the research vessel Alkor for assistance during the sampling.

7. References:
THU-AM-A6 Occurrence and spatial distribution of neutral per- and polyfluoroalkyl substances (n-PFASs) in sediments from Lake Shihwa, Korea in 2015 and 2021

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Introduction: Per- and polyfluoroalkyl substances (PFASs) are a group of chemicals with exceptional amphiphilic properties that have been used in various industrial and consumer applications as surfactants, grease repellants, waterproofing agents, and flame retardants since 1940s. Because of their bioaccumulation potential, toxicity, and ubiquitous presence, perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) have been listed as the persistent organic pollutants (POPs) under the Stockholm Convention since the 2000s. Consequently, neutral PFASs (n-PFASs) used as major intermediates in industrial sectors, such as fluorotelomer alcohols (FTOHs), perfluorooctane sulfonamides (FOSAs), and perfluorooctane sulfonamidoethanols (FOSEs), have been contaminants of emerging concern as precursors of PFOS and PFOA. Despite of growing evidence for the contribution of n-PFASs to ionic PFASs and their adverse health effects, research on environmental contamination profiles of n-PFASs are scarce as well as limited on air samples. In the present study, n-PFASs were measured in sediments from Lake Shihwa, Korea in 2015 and 2021 to investigate the occurrence, contamination status, and time trend.

Materials and Methods: Sediment samples were collected from the creeks, inshore, and offshore regions of Lake Shihwa, which is surrounded by intensively industrialized complexes, in 2015 (n=42) and 2021 (n=41). Fourteen n-PFASs were determined using solid-liquid extraction and simple clean-up with ENVI-carb cartridge followed by a gas chromatography-tandem mass spectrometry (GC-MS/MS).

Results: Among 14 n-PFASs, FTOHs were predominant compounds with over 95% contributions of total n-PFAS concentrations in all samples. The highest concentrations of n-PFASs were observed in creeks taken near industrial complexes. The concentrations of n-PFASs decreased with increasing distance from creeks to inshore and rarely detected in offshore. This tendency was found in both sediments from 2015 and 2021, although the total concentration of n-PFASs had declined by approximately three times in 2021 compared to 2015. Two sites with the highest levels of n-PFASs were found in creeks and considered as hot spot for PFASs contamination. Compared with our previous results on ionic PFASs in sediments from 2021, a similar spatial distribution and hot spots were observed. The investigation into fluorine related facilities in the Shihwa industrial complexes revealed that the manufacturing facilities for durable water repellents for textiles are located near these hot spots. The relative contribution of each FTOH to fluorotelomer substances in sediments was different between 2015 and 2021. The contribution of 6:2 FTOH increased from 5% to 31%, and the contribution of 8:2 and 10:2 FTOH decreased from 62% to 46% and from 30% to 23%, respectively.

Discussion and Conclusion: Our results suggest that geographical proximity to industrial sources is a major factor governing n-PFASs contamination in sediment, which would play a role as indirect and mobile sources of ionic PFASs. Fluorochemical consuming facilities, especially durable water repellents manufacturing, are potential contamination source of PFASs to near environment. Relative contribution in sediments indicate that n-PFASs usage patterns are shifting from long-chain (>C8 compounds) to short-chain (C6 compounds) based substances in fluorochemical industrial activity. Our findings provide a fundamental basis for the contamination profiles of n-PFASs in industrialized areas, and suggest that the comprehensive analysis of both neutral and ionic PFASs is essential to understand the environmental fate of PFASs on family concepts.

Acknowledgments: This work was supported by the projects entitled “Development of source identification and apportionment methods for toxic substances in marine environments” of Korea institute of Marine Science & Technology Promotion (KIMST-20220534) funded by the Ministry of Oceans and Fisheries (MOF), Korea.
Temporal trends and suspect screening of halogenated flame retardants and their metabolites in blubbers of cetaceans stranded in Hong Kong waters

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**Introduction:** Halogenated flame retardants (HFRs) are a large class of chemical additives intended to meet flammability safety requirements, and at present, they are ubiquitous in the environment. With the phasing out of legacy HFRs, many novel HFRs have emerged as replacements. Target analysis of HFRs usually focuses on a limited number of novel HFRs;[1] many HFRs have biotransformation potential, but HFR metabolites are less studied compared; all these can be partly attributed to the absence of relevant reference standards.[2] As top predators, marine mammals can bioaccumulate high amounts of persistent organic pollutants and are particularly susceptible to HFR exposure.[3] Thus, we conducted comprehensive target analysis and suspect screening of HFRs in blubber samples from 105 resident marine cetaceans stranded in Hong Kong waters between 2013 and 2020. We aimed to (1) investigate the pollution status of legacy and novel HFRs using cetacean blubber as the biomonitor, (2) examine the temporal trends of levels and composition profiles of these HFRs in these cetaceans, and (3) use high-resolution mass spectrometry (HRMS) to screen novel HFRs and potential HFR metabolites.

**Materials and Methods:** Twenty-six target legacy and novel HFRs were analyzed in this study. Blubber samples of finless porpoises (*Neophocaena phocaenoides*) (*n* = 70) and Indo-Pacific humpback dolphins (*Sousa chinensis*) (*n* = 35) were obtained. Target analysis of HFRs was performed using ultra-performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS). UPLC–quadrupole time-of-flight (QToF) HRMS was used for the suspect screening of HFRs, which was conducted using a suspect list that included three parts: (1) novel HFRs reported in recent years, (2) reported HFR metabolites, and (3) inferred HFR metabolites.[2] Eight common metabolic pathways were considered, including methylation, debromination, hydroxylation, dihydroxylation, methoxylation, demethylation, sulfation, and glucuronidation.[2]

**Results:** Target HFRs were found at µg/g lw levels in the blubber samples, revealing their high pollution burden. Polybrominated diphenyl ethers (PBDEs) and α-hexabromocyclododecane (α-HBCD) accounted for more than 95% of ΣHFRs. Significant decreasing temporal trends were observed in the concentrations of tetra-/penta-/hexa-BDEs in adult porpoises stranded in 2013–2015 than those stranded in 2016–2020. A significant positive correlation between these legacy and novel HFRs in the investigated cetaceans indicates their similar contamination source. 2,3-dibromopropyl-2,4,6-tribromophenyl ether (DPTE), a novel HFR, was found at high levels in the investigated marine cetaceans and accounted for more than 85% of the total novel HFRs. Semi-quantification results indicate the existence of methyl-methoxy-tetra-BDE (Me-MeO-tetra-BDE) at µg/g-level in the cetacean blubber samples.

**Discussion and Conclusion:** The declines of tetra-/penta-/hexa-BDE levels coincided with the phasing out of these PBDEs in China. No decreasing trend was observed in the levels of deca-BDE or HBCD, probably due to their exemption from the ban in China until 2025 and 2021, respectively. The current results provide a baseline for verifying if the ban of the exemption will be effective in lowering deca-BDE and HBCD in the future. A significant positive correlation was found between concentrations of tetra-BDE and Me-MeO-tetra-BDE, indicating that the metabolism of tetra-BDE may be a potential source of Me-MeO-tetra-BDE in marine mammals. Although the adverse effects of PBDEs have been revealed thoroughly by research studies in recent years, reports on the environmental occurrence, toxicokinetic and toxicodynamic characteristics of Me-MeO-tetra-BDE are very limited. More research efforts in these aspects are warranted.

**Acknowledgements:** This work was supported by the following research funds (shown with project numbers): 2022YFC3204800; ECF 2021-45; JCJ20190812155805559; SMSEGL20SC02; and 9648002. The authors thank the Agriculture, Fisheries and Conservation Department of the Hong Kong SAR Government for providing biosamples.

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1. Introduction:

Due to their high efficiency and low production costs, the use of brominated flame retardants (BFRs) in various materials and applications has steadily increased during the last 50 years [1, 2, 3]. With the increase in production and usage of BFRs, concerns about their environmental behavior grew. Nowadays, many conventional BFRs, like polybrominated diphenyl ethers and biphenyls (PBDEs and PBBs), hexabromocyclododecane (HBCDD) and tetrabromobisphenol A (TBBPA), are subject to close regulatory attention [4, 5]. With the phase-out of several legacy compounds, a new group of brominated organics, the so-called novel BFRs, were developed and placed on the market as drop-in substitutes. Like conventional BFRs, they are comprised of many different structures and have wide-spread applications [6]. However, as several physical-chemical properties of novel BFRs are similar to those of the persistent organic pollutants they replace (hydrophobicity, aromatic groups, bromine substitution), concerns for their environmental hazard profile have been expressed [3, 7]. To close the current gap of knowledge between conventional and novel BFRs, more studies on characteristics such as the bioaccumulation, mobility, degradation or toxicity of the substitutes are needed [3].

One special representative of the novel BFRs is “PolymericFR”, a polystyrene and brominated polybutadiene copolymer (Figure 1A) that is used as a safer alternative to HBCDD in polystyrene insulation foams. “PolymericFR” has a high molecular weight (20 to 50 times the size of conventional BFRs) and therefore possesses a low bioavailability. While the whole polymer has a large hydrophobic structure, it has been demonstrated that when “PolymericFR” is exposed to abiotic degradation processes like photolysis, it can be broken down into monomeric degradation products. These products are likely to be more mobile and bioavailable when compared to the parent polymer [8, 9]. However, currently there is only limited knowledge on changes in the environmental hazard profile of “PolymericFR” with respect to its abiotic degradation [9, 10, 11, 12].

In this study we examine the photolytic degradation of “PolymericFR” and compare it to the degradation of a monomeric alternative: Tetrabromobisphenol A-bis (2,3-dibrom-2-methyl-propyl) ether (TBBPA-BDBMPE; Figure 1B). While this TBBPA-Ether derivative is also a novel BFR, compared to “PolymericFR” it is structurally more closely related to legacy BFRs due to its monomeric nature and its structural basis of TBBPA. Therefore, TBBPA-BDBMPE can be seen as a common representative of most novel BFRs, while still having the same application in polystyrene foams as PolymericFR [13].

We investigate the photolytic degradation of both BFRs and the formation of degradation products. Additionally, we provide further insights into the changes of ecotoxicological effects with advancing photolytic degradation of the BFRs for two standard test organisms. Finally, we also examine how the storage of the photolysis samples affect the chemical composition and the ecotoxicological potential of the degradation mixtures.

2. Materials and Methods:

“PolymericFR” (CAS No. 1195978-93-8) and TBBPA-BDBMPE (CAS No. 21850-44-2) were separately irradiated in ultrapure water (30 min and 180 min UV exposure), simulating the exposure of the compounds to sunlight for up to 215 hours. The resulting degradation mixtures were filtered and then either used directly in chemical analysis and ecotoxicity testing, or stored for two weeks under different conditions (at room temperature or frozen) and subsequently used in the same analysis.
To investigate the formation of organic degradation products, high-resolution mass spectrometry (LC-HRMS) was conducted and a suspect screening was carried out. The screening covered 100 suspects identified from literature, including brominated as well as non-brominated compounds. Additionally, the HRMS data was used in a non-target analysis with Haloseeker2.0, a software that aims at identifying halogenated substances [14].

To examine ecotoxicological effects of the degradation mixtures, acute ecotoxicity testing with the green algae Desmodesmus subspicatus (OECD 201) and the water flea Daphnia magna (OECD 202) was conducted. No observed effect concentrations (NOECs) and, where possible, median effective concentrations (EC₅₀ values) were calculated and compared between the two BFRs, as well as for the different photolysis times and storage conditions.

3. Results:
Out of the 100 suspects, a total of 29 compounds were detected in the degradation mixtures of one or both BFRs. For both BFRs the number of detected molecular formulas, especially those of non-brominated compounds, increased with advancing photolysis (Figure 2). The comparison of the "PolymericFR" degradation mixtures to TBBPA-BDBMPE shows, that more non-brominated degradation products were found after the photolysis of the polymer. The different sample storage conditions influenced the number and bromination status of the degradation products for both BFRs, but the changes were more pronounced for the "PolymericFR" degradation mixtures. Generally, a greater number of highly brominated products with was detected when the samples were stored frozen for two weeks prior to LC-HRMS analysis.

The results of the non-target analysis confirmed the findings of the suspect screening with an increased detection of brominated clusters for both BFRs after longer photolysis and for those samples that were stored frozen. A maximum of 170 different brominated clusters were identified in the "PolymericFR" degradation mixtures, while more than 200 brominated clusters were identified in the TBBPA-BDBMPE degradation mixtures.

Figure 2: Number of detected molecular formulas of degradation products for the degradation mixtures of 30 and 180 min UV exposure of "PolymericFR" (A) and TBBPA-BDBMPE (B). The results are depicted separately for the three different storage conditions (no storage, storage at room temperature and frozen). The number of bromine atoms in the degradation products is indicated by the color of the bars.
The ecotoxicity results confirmed that the changes of chemical composition with advancing photolysis of the BFRs led to changes in the induced effects, which became more pronounced after longer UV exposure of the BFRs. Additionally, clear differences could be observed in the effects on the two test species. While algae growth was only slightly affected by the degradation mixtures (mostly below 10 % growth inhibition), an induction of up to 100 % immobility of daphnids could be observed for high concentrations of the 180 min UV exposed degradation mixtures of both BFRs. Additionally, the induction of daphnid immobility occurred at lower concentrations in those samples that had been stored frozen, suggesting an influence of sample storage on the ecotoxicological potential of the degradation mixtures.

4. Discussion:
4.1 Photolysis of "PolymericFR" and TBBPA-BDBMPE
Our results confirm that the exposure of "PolymericFR" and TBBPA-BDBMPE to UV light leads to the photolytic degradation of both BFRs. Of the eight brominated and eight non-brominated photolysis products previously reported for "PolymericFR" [8, 9], we have confirmed the formation of seven of the brominated and all of the non-brominated compounds. We have also identified six additional brominated degradation products in the suspect screening. The presence of even more unknown brominated products is suggested by the large number of brominated clusters we have detected in the degradation mixtures in the non-target screening. As no literature on the degradation behavior of TBBPA-BDBMPE is available, no comparison of our results to previous data was possible. However, we can conclude from our results that the formation of degradation products of both BFRs seems to be in the same order of magnitude. Concerning the ecotoxicological effects, our results show that the photolytic degradation of the BFRs alter their acute ecotoxicological potential towards the aquatic invertebrate D. magna. So far two studies have attempted to assess the ecotoxicity of "PolymericFR" degradation products to daphnids, one study by testing standards of four identified degradation products individually and in an artificial mixture [10] and the second study by applying in silico toxicity calculations for two degradation products [9]. Both studies conclude that the environmental hazard by the known degradation products is likely to be low. However, as discussed above, the actual number of degradation products can be assumed to be greater than the compounds included in these studies, and the occurrence of mixture effects can also not be ruled out. Therefore, we have decided to apply the complete degradation mixture in the ecotoxicity tests. Within this study, this approach led to considerable effects on the D. magna mobility, while the concentrations that we apply are far above the concentrations that we would expect in the environment. The ecotoxicity results of the present study also complement the finding of a previous study, where we saw no chronic effects of the degradation mixtures of both BFRs on the aquatic annelid Lumbriculus variegatus, but some evidence of acute physiological reactions of the annelid to those degradation mixtures that had been exposed to UV light for 180 min [12].

4.2 Influences of storage conditions on chemical composition and ecotoxicity
The storage conditions of the BFR photolysis samples altered the chemical composition of the degradation mixtures and subsequently, their effects on daphnids. The increased detection of highly brominated products, especially in the "PolymericFR" samples that had been stored frozen, may possibly be due to the occurrence of very large (possibly oligomeric) degradation products in the non-frozen samples. Such products could have escaped detection within the HRMS system, due to their size and lack of polarity. During the freezing or thawing process of the samples, such products could have been broken down, leading to their subsequent detection in those samples. Generally, the influence of storage conditions of water samples containing BFRs is thus far not well studied. Within the broader field of halogenated organics, storage conditions have thus far mainly been investigated for organochlorine pesticides [15].

5. Conclusions:
Our study provides insights into the degradation behavior and subsequent changes in ecotoxicological potential for two novel BFRs used in the same application. It therefore enables a comparison of the polymeric and monomeric BFR alternatives. However, further studies are needed to completely postulate the degradation mechanisms of the BFRs. To finalize a risk assessment of the compounds, additional data on their environmental exposure is needed, therefore the novel BFRs and their degradation products should be included in future measurement campaigns. Since the stability of analytes is a prerequisite for reliable exposure measurements, more studies on the influences of storage conditions on BFRs in water samples are also needed.

6 References:
THU-AM-B3  Polybrominated Diphenyl Ethers Disrupt Visual Perception and Correlated Central Nervous Functions of Zebrafish Larvae

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Introduction: Visual system is a remarkable information sensor for the central nervous system. The newly discovered non-image-forming (NIF) visual system is dependent on intrinsically photosensitive retinal ganglion cells (ipRGCs), which employs melanopsin as the signaling photopigment and performs NIF functions of, for example, circadian rhythm, sleep, and mood. Some studies reported that polybrominated diphenyl ethers (PBDEs), a conventional category of brominated flame retardant additives, could possibly impair the structure and functions of visual system in zebrafish larvae, however, the underlying mechanism and related retina-brain neurotoxic pathway is yet to be investigated.

Materials and Methods: Choosing BDE-47 and BDE-99 as representatives of PBDEs, we exposed wildtype zebrafish embryos for 144 hours. For effects concerning image-forming vision, the changes after BDE-99 exposure were examined in retinal histological structure, relevant gene expression, and vision-guide behaviors. We further used thyroid hormone (TH) antagonist to explore the roles of TH signaling in the visual toxicity of BDE-99. For effects concerning non-image-forming vision, two time points were scheduled in the experiment of BDE-47 exposure including 9 a.m. at 6 dpf and 11 p.m. at 5 dpf, respectively. Quantitative real-time PCR and in situ hybridization together located and quantified the expression of melanopsin genes in zebrafish larvae, as well as key genes in circadian and 5-HT system. Immunofluorescent staining was used to label suprachiasmatic nucleus (SCN) neuropeptides and hypothalamic 5-HT. The depression-like phenotype and circadian disruption of larvae were characterized using thigmotaxis and sleep/wake behavior tests.

Results: After BDE-99 exposure, the thickness of the retinal photoreceptor cell layer was reduced, and the opsin expression exhibited an interesting disturbed pattern that only the longest wavelength opsin (opn1lw1) was significantly upregulated, which was consistent with color-vision based optokinetic response. The results of T3 administration indicated that TH signaling was not the primary mechanism for BDE-99’s visual toxicity. We then found six7, a transcription factor that plays an important role in photoreceptor patterning, was the actual contributor by six7 expression modulation. Furthermore, BDE-47 caused disruptions with a distinct day-night pattern on the expressions of photopigment melanopsin that dominates the NIF visual system. Such bidirectional difference transmitted to clock genes and neuropeptides in the SCN and impacted adjacent serotonin system. However, indicative factors of depression including serta and aanat2 were unidirectionally increased probably due to the time-specific roles of melanopsin. They were consistent with changes of nighttime thigmotaxis and sleepy hypoactivity.

Discussion and Conclusion: The visual system was a sensitive target of PBDEs compared with conventional neurotoxic endpoints. Although PBDEs were well-known as TH disruptors and BDE-99 exerted a TH-like effect on the photoreceptor patterning, we provided a new toxicological interpretation and the key point in the events was BDE-99 suppressed the expression of six7. Besides, the impacted melanopsin by BDE-47 further altered clock genes, neurotransmitters, hormones, and metabolic enzymes in various brain regions by means of ipRGC projections, and caused depression-like behaviors in zebrafish larvae. It is an extension to the works regarding classical image-forming vision, suggesting the visual impairments of PBDEs probably have profound and complex health implications including various higher cognitive and emotional functions of human brain as recent scientific progress claimed.

Acknowledgments: This work was funded by the National Natural Science Foundation of China (22236006).

References:
Legacy and Emerging Flame Retardants
K. Fernie & J. De Boer

THU-AM-B4  Atmospheric Deposition of Hexabromocyclododecane in the Urban Background of Bavaria, Germany

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Introduction: Hexabromocyclododecane (HBCD) has been widely used from the 1960s until 2017 as additive flame retardant primarily in polystyrene foams (EPS, XPS) in heat insulation systems for buildings. Due to its persistence, bioaccumulation and toxicity HBCD was listed as POP under the Stockholm convention in 2013 and phased out in the European Union in 2017 by the REACH regulation. Total HBCD consumption in Germany between 1966 and 2016 is estimated to 42,800 t in EPS and 19,100 t in XPS [1] covering surfaces of a around 1000 km². Since the maximum of HBCD containing waste is expected to occur around 2050 [1] diffuse release from insulation systems into the atmosphere will continue for decades. However, direct quantification of HBCD emission into the atmosphere is virtually impossible. Therefore, we measured atmospheric deposition rates of HBCD at a representative monitoring station in the urban background for one year and made a backcalculation of its atmospheric release for the state of Bavaria, Germany.

Materials and Methods: Atmospheric bulk deposition of HBCD was measured between March 2021 and February 2022 by the funnel-adsorber method standardized for chlorinated dioxins/furans (guideline VDI 2090 part 2) beneath the air monitoring station at the Bavarian Environment Agency (LfU) in the urban background of the city of Augsburg. Since no building with PS insulation system is in direct neighbourhood, the sampling location is not impacted by a HBCD point source and thus can be regarded as representative for the urban background. The funnel-adsorber sampler consists of a glass funnel with 0.25 m diameter fixed at a height of 1.5-2 m above ground and connected at the bottom to a glass cartridge filled with precleaned XAD-2 resin covered with glass wool. Two sampling units were operated in parallel. The cartridges were changed after 30 days. XAD-2 resin and glass wool were extracted in a soxhlet apparatus with n-heptane/acetone-(1:1) after addition of 13C12-labeled γ-HBCD. After clean-up with silica/44 % conc. sulfuric acid and a florisil column the sum of HBCD isomers was determined by gas chromatography coupled to tandem mass spectrometry (GC-MS/MS). A field blank as well as a laboratory blank sample was processed together with each pair of deposition samples. In one case with a quantifiable HBCD blank the twofold of the blank was used as limit of quantification (LOQ) according to guideline VDI 2464 part 3.

Results: The analysis of 13 deposition samples from 7 monthly periods revealed a range of HBCD deposition rates from < LOQ (LOQ = 0.54 ng/(m²*d)) to 10.6 ng/(m²*d) with an arithmetic mean value of 2.53 ng/(m²*d) whereby half of the LOQ was used for results below LOQ. This deposition rate was considered as representative for the whole settlement and transport area in Bavaria covering 12 % of the surface (8536 km²). For the rural and remote areas (88 % of the surface) deposition rates are assumed to be considerably lower but due to the much larger area we conclude that total deposition of HBCD on rural and remote areas is about equal to deposition on the settlement and transport area. Thus, total HBCD emission into the atmosphere in Bavaria is two times higher than measured deposition on the settlement and transport area and is calculated to 16 kg per year.

Discussion and Conclusion: The HBCD emission is in the same order of magnitude than calculation with the measured HBCD release rate from an emission chamber of 6.5 ng/(m²*h) [2]. A lower HBCD release of 0.79 kg/year is calculated using an annual emission rate from PS foams of only 8.4*10⁻⁶ % estimated by ECHA’s chemical safety report on HBCD. On the other hand a higher atmospheric HBCD emission of 30 kg/year was estimated for Switzerland although the number of inhabitants is lower than in Bavaria [3]. Our calculation only considers current HBCD inventories as emission sources but not end of life emissions occurring during renovation, demolition and recycling that will be more important in future.

References:
Brominated Flame Retardants in Plastic Products from China, Indonesia, Russia, and the Philippines

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1. Introduction:
Brominated flame retardants (BFRs) are artificial chemicals commonly added to various products to prevent a fire. The frequent fires due to cigarettes at home in the 1970s triggered the production and use of BFRs1. The solution leads to the birth of chemicals known as fire retardants rather than responses to increasing the fire safety of cigarettes. Since then, brominated flame retardants have been used in various products, especially in electronics, home and car furniture, upholstery, matrasses, textiles, and insulations2.

BFRs include several different types of chemicals, such as polybrominated diphenyl ethers (PBDEs), hexabromocyclododecanes (HBCDs), and tetrabromobisphenol A (TBBPA). They are typically used in acrylonitrile butadiene styrene (ABS) plastics, polyurethane (PU) foams, and polystyrene (PS) plastics. These materials are the main components for producing toys, electronic casings, textiles, furniture uphosterling, and building insulations. BFRs could be released from the products when consumers use these products3,4. Furthermore, other harmful brominated substances, such as brominated dioxins (PBDD/Fs), occur as unintentional by-products of BFR applications5.

TBBPA is the flame retardant produced in the most significant volumes. TBBA, PBDEs, and HBCD are endocrine-disrupting chemicals6,7 which decompose very slowly under natural conditions and can travel far from their origin through water and air currents8,9. Previous studies have shown the presence of PBDEs and HBCD in new products and household equipment10,11, including children’s toys12-14, thermo cups, kitchen utensils15, office utensils16, hair accessories16,17, and carpet padding18,19.

The current study aims to determine whether children’s toys, hair accessories, office supplies, kitchen utensils and other consumer products found in the Chinese, Indonesian and Russian markets are still affected by the same poor practice of plastic recycling. Similar studies were conducted in these countries in 2015–201812,17,20, so this new research is also an opportunity to look at potential trends in levels of BFRs in consumer products made of recycled black plastic. It is also an opportunity to generate the first data about levels of TBBPA in the studied products. A previously published report contains a more detailed description of the samples.21

2. Materials and Methods:
In October–December 2020, we purchased 455 samples of consumer products made of black plastics from markets and stores in China, Indonesia, and Russia. Electronic casings are typically black and generate black plastics when recycled. We choose products in black colors that are not required to meet fire standards deliberately. So, we assumed that any BFRs present were not added to the product but rather because of recycling plastics containing BFRs. Children’s toys, hair accessories, kitchen utensils and office supplies were of primary interest because they are used by children and women of reproductive age who are potentially at risk of BFR exposure22,23. X-ray fluorescence was used to determine bromine content which indicates the presence of BFR in plastics24 and to do a preliminary screening of the plastics using a handheld NITON XL 3t 800 XRF analyzer. We chose samples containing >213 ppm of bromine and >64 ppm of antimony for further analysis. These screening criteria were applied since bromine is a key component of BFRs, and antimony trioxide is a common BFR synergist25.

We also chose samples to cover all three countries and all sample categories (toys, office supplies, hair accessories, kitchen utensils, and other items). Of the 455 samples, 73 were selected for lab analysis: 30 from Russia, 20 from China, and 23 from Indonesia. In addition, we added another three samples selected similarly in the Philippines for BFRs analyses to this collection from China, Indonesia, and Russia. All these 76 samples were analyzed for 16 PBDE congeners. For purposes of calculation, the components of the commercial PentaBDE mixtures include congeners BDE 28, 47, 49, 66, 85, 99, 100, and OctaBDE mixtures contain the following congeners: BDE 153, 154, 183, 196, 197, 203, 206, 207. The component of the commercial DecaBDE mixture is BDE 209.
MORNING BREAKOUT SESSIONS
THURSDAY 14 SEPTEMBER 2023

➤ 10:00 - 11:50

Legacy and Emerging Flame Retardants
K.Fernie & J.De Boer

THU-AM-B6  Brominated Flame Retardants in Plastic Products from China, Indonesia, Russia, and the Philippines

Three isomers of HBCD (-, -, -HBCD), TBBPA, and six nBFRs, i.e., 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE), decabromodiphenyl ethane (DBDPE), hexabromobenzene (HBB), octabromo-1,3,3-trimethylphenyl-1-indane (OBIND), 2,3,4,5,6-pentabromobenzylbenzene (PBEB), and pentabromotoluene (PBT) were also analyzed in the samples. Targeted BFRs were isolated by the triple ultrasonic extraction using n-hexane: dichloromethane (4:1, v/v). Identification and quantification of PBDEs and nBFRs were performed using gas chromatography coupled with mass spectrometry in negative ion chemical ionization mode (GC-MS-NICI). Identification and quantification of HBCD isomers and TBBPA were performed by liquid chromatography interfaced with tandem mass spectrometry with electrospray ionization in negative mode (UHPLC-MS/MS-ESI-). The limit of quantitation was 1 ng/g for BDE 206, 207 and 209 and 0.5 ng/g for 13 others analyzed PBDE congeners, ranged between 0.5-5 µg/kg for nBFRs, and was 0.5 µg/kg for HBCD and 5 µg/kg for TBBPA.

3. Results:
The results of analyses for BFRs in 76 samples analyzed in this study are summarized in Tables 1 and 2.

Table 1: PBDEs and HBCD levels measured in samples from China, Indonesia, Russia, and the Philippines. Amounts in mg/kg (ppm).

<table>
<thead>
<tr>
<th>Country</th>
<th>N</th>
<th>PentaPBDE</th>
<th>OctaPBDE</th>
<th>DecaPBDE</th>
<th>PBDEs</th>
<th>HBCD</th>
</tr>
</thead>
<tbody>
<tr>
<td>China</td>
<td>N=20</td>
<td>&lt;LOQ</td>
<td>0.029 – 100</td>
<td>&lt;LOQ – 316</td>
<td>0.023 - 366</td>
<td>&lt;LOQ - 4.66</td>
</tr>
<tr>
<td>Russia</td>
<td>N=30</td>
<td>&lt;LOQ</td>
<td>0.84 – 125</td>
<td>1.91 - 443</td>
<td>2.75 – 497</td>
<td>&lt;LOQ - 3.97</td>
</tr>
<tr>
<td>Indonesia</td>
<td>N=23</td>
<td>&lt;LOQ - 1.78</td>
<td>0.008 – 262</td>
<td>0.088 - 256</td>
<td>0.10 – 405</td>
<td>&lt;LOQ - 1.51</td>
</tr>
<tr>
<td>Philippines</td>
<td>N=3</td>
<td>&lt;LOQ</td>
<td>57 - 128</td>
<td>76 - 124</td>
<td>133 - 252</td>
<td>0.16 – 7.28</td>
</tr>
</tbody>
</table>

Table 2: TBBPA and novel BFRs (nBFRs) levels measured in samples from China, Indonesia, Russia, and the Philippines (in mg/kg).

<table>
<thead>
<tr>
<th>Country</th>
<th>TBBPA</th>
<th>BTBPE</th>
<th>DBDPE</th>
<th>HBBz</th>
<th>OBIND</th>
<th>PBEB</th>
<th>PBT</th>
<th>Sum of nBFRs</th>
</tr>
</thead>
<tbody>
<tr>
<td>China</td>
<td>0.3 0 - 290</td>
<td>&lt;LOQ-557</td>
<td>&lt;LOQ-75</td>
<td>0.014-1.14</td>
<td>&lt;LOQ-422</td>
<td>&lt;LOQ</td>
<td>0.003-0.48</td>
<td>5.37-728</td>
</tr>
<tr>
<td>Russia</td>
<td>0.3 2 - 368</td>
<td>0.10-557</td>
<td>5.37-83</td>
<td>0.003-0.44</td>
<td>0.38-75</td>
<td>&lt;LOQ</td>
<td>0.002-0.43</td>
<td>5.85-655</td>
</tr>
<tr>
<td>Indonesia</td>
<td>0.3 0 - 268</td>
<td>0.09-389</td>
<td>&lt;LOQ-66</td>
<td>0.001-0.34</td>
<td>&lt;LOQ-18</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ-0.09</td>
<td>1.81-408</td>
</tr>
<tr>
<td>Philippines</td>
<td>154-374</td>
<td>185-315</td>
<td>38-63</td>
<td>0.402-3.78</td>
<td>31-79</td>
<td>&lt;LOQ</td>
<td>0.10-0.72</td>
<td>256-435</td>
</tr>
</tbody>
</table>

Figure 1: Examples of products analyzed in this study (n=455).
Legacy and Emerging Flame Retardants
K. Fernie & J. De Boer

THU-AM-B6  Brominated Flame Retardants in Plastic Products from China, Indonesia, Russia, and the Philippines

All samples contained octaBDE at concentrations ranging from 0.008 to 261.7 ppm and 72 samples contained decaBDE at concentrations ranging from 0.088 to 442.6 ppm. HBCD and pentaBDE were only detected at very low concentrations, which is expected since these flame retardants are primarily used in polystyrene insulation and foam products and not in electronic casings. None of the samples were required to meet any fire safety standards. In addition, the measured levels of BFRs do not provide a fire-retardant function. Therefore, it is likely that the BFR content comes from recycled e-waste plastics. BTBPE, DBDPE, and OBIND showed the highest values of nBFRs in the analyzed products from four countries in this study.

4. Discussion:
Table 3 shows that the concentrations of octaBDEs in the samples of children’s toys were at the same levels as in toys sampled from China and Indonesia for this study as well as in those from the study of toys collected from 26 countries in 2017.12 However, octaBDE levels in toys from Russia were higher in samples from 2017 than in 2020. DecaBDE concentrations were higher in this study so we can see an increasing trend of decaBDE levels in toys from recycled black plastic. However, this trend seems to be different from what was found in similar samples from Czechia and Serbia.14 Levels of nBFRs increased in toys from China, Indonesia (Table 3), Czechia and Serbia14 between the years 2017 and 2020 while they were found to decrease in samples from Russia. We found these trends among a limited number of analyzed products/toys. For example, TBBPA showed higher levels in samples from this study than those from Czechia and Serbia collected in 2020. Still, they were much lower compared to the samples from 2018 from two central European countries.14

Table 3: Comparison of PBDE Concentrations in Children's Toys between 2017 and 2020 (in mg/kg)

<table>
<thead>
<tr>
<th>Country</th>
<th>OctaBDE Year 2017</th>
<th>OctaBDE Year 2020</th>
<th>DecaBDE Year 2017</th>
<th>DecaBDE Year 2020</th>
<th>nBFRs Year 2017</th>
<th>nBFRs Year 2020</th>
</tr>
</thead>
<tbody>
<tr>
<td>China</td>
<td>3 - 58</td>
<td>5 - 94</td>
<td>2 - 36</td>
<td>23 - 136</td>
<td>76 - 117</td>
<td>5.37 - 728</td>
</tr>
<tr>
<td>Indonesia</td>
<td>&lt;LOQ - 52</td>
<td>&lt;LOQ - 63</td>
<td>0.008 - 71</td>
<td>0.09 - 256</td>
<td>2.7 - 452</td>
<td>5.85 - 655</td>
</tr>
<tr>
<td>Russia</td>
<td>1 - 362</td>
<td>0.84 - 125</td>
<td>&lt;LOQ - 217</td>
<td>1.91 - 304</td>
<td>&lt;LOQ - 714</td>
<td>1.81 - 408</td>
</tr>
</tbody>
</table>

All four countries in this study are both producers and potential recipients of e-waste containing POP-BFRs. To stop imports of waste with POP-BFRs, strict limits for POPs content in waste need to be established. The 2022 Conference of Parties to the Basel and Stockholm Conventions suggested using either a 50 ppm, 500 or 1,000 ppm limit for POPs waste containing PBDEs25 (the so-called "low POPs content" level). With the weaker limit of 1,000 ppm, all wastes containing less than 1,000 ppm of PBDEs will be considered "clean" and allowed for export for recycling or disposal. This weak, "low POPs content" level raises concerns since PBDEs are very similar in structure and toxicological profiles to the highly toxic polychlorinated biphenyls (PCBs)27,28. The POPs content level for PCBs in waste under the Conventions is 50 ppm and it would therefore be consistent for PBDEs also have a 50 ppm limit26. Of the analyzed products in this study 65 out of 76 (85.5 %) would be categorized as POPs waste using a 50-ppm limit.

Moreover, a weak "low POPs content" level above 50 ppm would lead to decreasing demand for superior waste disposal technologies with the ability to fully destroy BFRs in the waste while not emitting any unintentionally produced POPs (U-POPs). Truly environmentally sound BFR destruction technologies exclude incineration processes. Although Russia and China have the technical capability and pilot plants, they have not yet invested sufficiently to establish commercial non-combustion plants for POP destruction. The Philippines uses non-combustion plants for PCBs destruction.

It is also important to note that 5, 6 and 7 samples presented in this study from China, Indonesia and Russia respectively were recently analyzed also for the content of brominated dioxins (PBDD/Fs) and dioxin-like activity with DR CALUX bioassay analysis. The results were in the range of 183 - 4,580 pg WHO-TEQ/g dw, and 110 – 13,680 pg BEQ/g dw for PBDD/Fs and DR CALUX respectively26. It confirms that very toxic POPs unintentionally produced during PBDEs production and or during recycling of e-waste plastics accompany BFRs carried into new consumer products5,29 of sensitive use and suggests being set rather strict LPCL in order to avoid potential risk for children and women.

5. Conclusions:
This study shows that children’s toys, hair accessories, office supplies and kitchen utensils in the Chinese, Indonesian, Russian, and Philippine markets contained brominated flame retardants (BFRs). The BFRs likely originated from unregulated recycling of e-waste plastics as all four countries are all producers and importers of e-waste potentially containing POP-BFRs. This practice contaminates and compromises a circular plastic economy, which means that the production of plastics containing hazardous
chemicals cannot continue. None of these countries has regulations limiting BFR content in products or waste. Applying a class-
based approach that restricts the use of all POP-BFRs, including regrettable substitutes currently used in products in the targeted
countries without any regulation, monitoring or control, would significantly contribute to an increased circularity. Also, existing
contaminated materials must be separated from the waste stream and POP-chemicals destroyed or irreversibly transformed
to stop the further spreading of POP-BFRs. Therefore, one crucial initial step towards a non-toxic circular economy is to set a
strict, low POPs content limit for wastes. This limit should be set at a concentration that prevents the recycling of POP-BFRs and
unintentionally produced PBDD/Fs accompanying them into new products and stops the export of POP-BFR contaminated wastes
into developing and transition countries.

6. Acknowledgments:
Research of the products in China, Indonesia and Russia was conducted with financial support from the Swedish Environmental
Protection Agency, and the work on this short paper was also supported by the Global Greengrants Fund.

7. References:
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toxic BFRs in toys, kitchen utensils and other consumer products from plastic in Czechia and Serbia. Organohalogen Compd.
THU-AM-B6  Brominated Flame Retardants in Plastic Products from China, Indonesia, Russia, and the Philippines

1. Introduction:
The Environment Protection Agency of Apulia has performed several measurement of persistent organic pollutants (POPs) stack emissions for a number of industrial plants located in a large industrial area near Taranto starting from 2007 [1]. Estimates of yearly mass flows for polychlorinated dibenzodioxins and dibenzofurans (PCDD/PCDF) and polychlorinated biphenyls (PCBs) from integrated steelwork were already available and published in relevant European inventories since the year 2000 [Reference missing]. Still, the actual measurements were in exceedance of those estimates and prompted the need of a more in-depth investigation of the fate and transport of POPs from emissions and release to the various environmental compartments. Indeed, in early 2008 high levels of dioxin and dioxin-like PCBs were found in food samples of animal origin that were collected from farms located in the immediate surroundings of the industrial area, causing immediate alarm among citizens and authorities [2]. As an immediate response to the events, as soon as December 2008 a regional regulation (LR 44/2008) enforced more stringent limits on dioxin stack emissions for plants operating in the metal sector. In fact, the existing Italian emission limits were inadequate to prevent POPs accumulation in the environment and not aligned to relevant regulation already into force in other European countries. In addition, and in order to investigate the extent of the contamination, an extensive monitoring plan was set up and performed throughout the period 2008-2011 [3]. As a result of the monitoring the local health autohority enforced the ban on grazing over wasteland within 20 km from the industrial area and a ban on the consumption of goat and sheep liver originating from animals grown in the same area, together with the stamping of over 2000 sheep and goats. A first result of all of the above actions has been the enforced reduction of the yearly mass flow of POPs emitted to the atmosphere as stack exhaust gases, as demonstrated by the latest emission measurements [4]. Nevertheless, also diffuse and fugitive emissions are contributing to the overall impact on the surroundings resulting in a measurable POPs atmospheric deposition of both wet and dry particles on soils and other urban surfaces [5,6]. This paper presents the results of PCDD/F and PCBs monitoring in stack emissions, soils and atmospheric deposition in the Taranto area during a thirteen-year period as a tool for assessing PCDD/F fallout in nearby urban-industrial areas.

2. Materials and Methods:
Bulk atmospheric deposition was collected according to method ISTISAN 06/38 e UNI EN 15980:2011 by using funnel-bottle bulk collectors consisting of a cylindrical funnel and a 10-liter sample collection vessel, both made of glass and left permanently open to the atmosphere with the funnel opening. The area of the cross-section of the funnel opening has a surface of 0.03731 square meters and the sampling time is 30 ± 2 days having been positioned at about 1.8 meters above ground. Extracts aliquots for the determination of PCDD/Fs and PCBs were obtained according to method UNI EN 16691:2015 (and EN 16693:2051, EN 16694:2015) using solid phase extraction (SPE) with SPE-disks and were purified/fractionated by means of an automated clean-up process using disposable columns (multilayer silica, alumina and carbon). Aliquots for the determination of PAHs were purified over silica gel. Compounds of interest were separated by high resolution gas chromatography (HRGC) on a capillary column (60 m x 0.25 mm, 0.25 µm film thickness). Isotope-dilution high-resolution mass-spectrometry determinations (HRMS) were carried out on a High Resolution magnetic-sector instrument at a resolution of 10000 operating with electron ionisation (EI) at 45 eV in the selected ion monitoring (SIM) mode. PAHs were separated by HRGC and determined by HRMS, in the same operating conditions used for PCDD/Fs. For each batch of twelve samples, a laboratory blank and a control sample were analysed. In addition, every sampling campaign included a field blank, that was used to ensure that no contamination had occurred during all steps of the measurement, and were not subtracted from measured values. Recovery rates for sampling and extraction labelled standards were in compliance with requirements (grater than 50%). Toxic equivalents (TEQ) for PCDD/Fs and PCBs were calculated using WHO toxic equivalency factors (WHO-TEFs, 2005). The laboratory has ISO17025 accreditation.

3. Results and Discussion:
The location of the sampling stations is depicted in figure 1 and shows how a transect from sources to the farthest sampling station (almost 10 km away from the industrial area, on the left of the map) was chosen in order to evaluate the fallout according to the distance. The results for the thirteen year period are presented in Table 1 and graphically represented in Figure 2, where it can be seen how a clear reduction of the atmospheric deposition of dioxin-like compounds have been observed around year 2013. Reasons for this improvement can be found on enforcement of stricter control and new legislation, together with investments and improved process management at the source level, although a reduction in overall production of goods as a consequence of a lessened market demand for finished products in the relevant industrial sectors might be invoked.
THU-AM-C1  Long term monitoring of wet and dry atmospheric depositions as a tool for assessing PCDD/F fallout in proximate urban-industrial areas

Figure 1: Location of bulk samplers locations

Table 1: Yearly amounts of dioxin-like compounds (PCDD/F and dl-PCBs). Amounts in pg TEQ/m² per day

<table>
<thead>
<tr>
<th>Year</th>
<th>TAMBURI CHIESA</th>
<th>TAMBURI ORSINI</th>
<th>DELEDDA</th>
<th>PREFETT.</th>
<th>CARMINE</th>
<th>TALSANO</th>
<th>AGL2</th>
</tr>
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<tbody>
<tr>
<td>2008</td>
<td>24,1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>13,92</td>
<td>6,89</td>
<td>-</td>
</tr>
<tr>
<td>2009</td>
<td>15,7</td>
<td>-</td>
<td>-</td>
<td>7,15</td>
<td>8,61</td>
<td>5,74</td>
<td>-</td>
</tr>
<tr>
<td>2010</td>
<td>13,3</td>
<td>-</td>
<td>-</td>
<td>3,70</td>
<td>9,22</td>
<td>7,81</td>
<td>-</td>
</tr>
<tr>
<td>2011</td>
<td>19,9</td>
<td>-</td>
<td>-</td>
<td>3,71</td>
<td>8,25</td>
<td>2,78</td>
<td>-</td>
</tr>
<tr>
<td>2012</td>
<td>19,9</td>
<td>-</td>
<td>10,31</td>
<td>3,35</td>
<td>5,26</td>
<td>3,92</td>
<td>-</td>
</tr>
<tr>
<td>2013</td>
<td>8,6</td>
<td>-</td>
<td>6,40</td>
<td>-</td>
<td>2,15</td>
<td>1,60</td>
<td>-</td>
</tr>
<tr>
<td>2014</td>
<td>2,9</td>
<td>-</td>
<td>6,45</td>
<td>-</td>
<td>0,98</td>
<td>0,89</td>
<td>-</td>
</tr>
<tr>
<td>2015</td>
<td>3,4</td>
<td>-</td>
<td>4,12</td>
<td>-</td>
<td>2,34</td>
<td>1,62</td>
<td>-</td>
</tr>
<tr>
<td>2016</td>
<td>1,9</td>
<td>4,0</td>
<td>2,00</td>
<td>-</td>
<td>1,48</td>
<td>0,46</td>
<td>9,96</td>
</tr>
<tr>
<td>2017</td>
<td>-</td>
<td>5,2</td>
<td>2,08</td>
<td>-</td>
<td>0,77</td>
<td>0,69</td>
<td>8,52</td>
</tr>
<tr>
<td>2018</td>
<td>-</td>
<td>5,7</td>
<td>3,01</td>
<td>-</td>
<td>5,80</td>
<td>0,99</td>
<td>9,95</td>
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<tr>
<td>2019</td>
<td>-</td>
<td>4,3</td>
<td>0,89</td>
<td>-</td>
<td>1,43</td>
<td>0,92</td>
<td>23,00</td>
</tr>
<tr>
<td>2020</td>
<td>-</td>
<td>2,0</td>
<td>0,75</td>
<td>-</td>
<td>1,10</td>
<td>2,25</td>
<td>16,71</td>
</tr>
<tr>
<td>2021</td>
<td>-</td>
<td>2,9</td>
<td>4,05</td>
<td>-</td>
<td>3,18</td>
<td>0,62</td>
<td>12,31</td>
</tr>
</tbody>
</table>
THU-AM-C1  Long term monitoring of wet and dry atmospheric depositions as a tool for assessing PCDD/F fallout in proximate urban-industrial areas

Figure 2: Time trends dioxin-like compounds on a yearly basis

Data at the monthly scale are of particular interest in order to follow time trends with greater detail since they are able to show acute deposition events. The monthly averages for the position named Carmine, a farm were contamination of sheep and goats above maximum values was detected, show how peaks of dioxin can still be seen in recent times, for periods spanning from 1-2 to 3-4 consecutive months (Figure 3).

Figure 3: Time trends of dioxin and dioxin-like compounds on a monthly basis. Amounts in pg TE/m² per day.
THU-AM-C1  Long term monitoring of wet and dry atmospheric depositions as a tool for assessing PCDD/F fallout in proximate urban-industrial areas

5. Conclusions:
Studies are still ongoing to evaluate if the improvements in stack emissions are having a concomitant positive effect on the observed POPs levels in the surrounding environment. Also, statistical investigations are being carried out using congener patterns in order to trace the sources of the possible contamination.

6. References:
The concentration of PCDD/Fs, PCBs and PCNs in PM$_{2.5}$ in coal-fired sampling site Taiwan in winter (9.01, 0.12, and 0.098 fg TEQ WHO/m$^3$, respectively) were greater than summer (2.88, 0.05, and 0.017 fg TEQ WHO/m$^3$, respectively). The congener profiles of PCDD/Fs in coal-fired sampling site were showed in Fig 2(a), 1,2,3,4,6,7,8-HpCDF, OCDF, 1,2,3,4,6,7,8-HpCDD and OCDD were dominated in congener profiles. PCDD/PCDF ratio in this study was between 0.098-0.39 indicated that the source might from thermal process. The congener profiles of PCBs in coal-fired sampling site were showed in Fig 2(b), TeCB77, PeCB118 and PeCB105 were dominated in PCBs congener profiles. The congener profiles of PCNs in coal-fired sampling site was showed in Fig 2(c), HeptaCN and OctaCN were dominated in winter, and low chlorine PCNs were dominated in other seasons. The study indicates that the high proportion of SO$_4^{2-}$, NH$_4^+$, NO$_3^-$, and Cl$^-$ in fine particles is associated with vehicle exhaust emissions, while SO$_4^{2-}$, NH$_4^+$, NO$_3^-$, Na$^+$, and K$^+$ are the main species emitted from waste incineration plants. SO$_4^{2-}$ and NH$_4^+$ are also associated with emissions from coal-fired boilers. The figure shows that NH$_4^+$ has a particularly high proportion during winter, which may be influenced by monsoon events.
The S.O.R. and N.O.R. ratios can be used to understand the degree of transformation of SO₂ and NOₓ into sulfates and nitrates in the atmosphere, as well as the transport of chemical species. The study calculated the S.O.R. and N.O.R. ratios in the central zone. The S.O.R. ratio was higher than 0.1 and greater by 0.25 during winter samples, indicating that in addition to local pollution sources, winter is also influenced by external events. In gas-fired sampling site, during the summer and winter seasons, the average concentrations of total water-soluble ion were 3.36 µg/m³ ± 0.59 µg/m³, and 5.43 µg/m³ ± 0.90 µg/m³, respectively. In the summer, negative values were observed for Mg²⁺, which could be attributed solely to the contribution from sea salt after calculating the non-sea salt magnesium ion concentration. The compositions of water-soluble ion are shown in Figure 4. It shown that nss-SO₄²⁻ had the highest concentration at gas-fired sampling site in both two seasons, and nss-SO₄²⁻, NO₃⁻, and NH₄⁺ are considered secondary inorganic aerosols (SIAs) that mainly originate from anthropogenic pollution, suggesting that monitoring station in both seasons were affected by anthropogenic pollution. The SIAs accounted above 70% of the ions at gas-fired sampling site, indicating that the local ions were mainly derived from secondary aerosols. The distribution of species in summer with high proportions of nss-SO₄²⁻, NH₄⁺, and NO₃⁻, possibly due to anthropogenic activities such as vehicle exhaust and industrial combustion emissions (Ali et al. 2019). The concentration of water-soluble ions in winter was also nss-SO₄²⁻, NH₄⁺, and NO₃⁻ remaining the main contributors. Though the distribution of nss-SO₄²⁻ was the highest in gas-fired sampling site, the concentrations of nss-SO₄²⁻ were lower than coal-fired sampling site due to the lower concentration of water-soluble ions.

The result of PMF of PCDD/Fs was showed in Fig 5(a), PCDD/Fs were divided into 5 factors as medical waste incinerator (MWI) (6.06%), Electric arc furnace (EAF) (63.9%), traffic emission (16.9%), secondary zinc smelter (10.7%) and crematorium (2.51%). The main species in factor 1 were OCDD (68.1%), in factor 2 was 2,3,4,7,8-PeCDF (58.3%), in factor 3 were OCDD (25.4%) and 1,2,3,4,7,8-HxCDF (17.9%), in factor 4 was 2,3,4,7,8-HxCDF (23.4%), and factor 5 were 1,2,3,7,8,9-HxCDF (14.2%) and 2,3,4,6,7,8-HxCDF (14.3%). The result of PMF of PBs was showed in Fig 5(b), PBs were divided into 3 Factors as MWI (21.2%), woodchip boiler (35.1%) and EAF (13.8%). The main species in factor 1 were PeCB-118 (41.6%) and PeCB-105 (19.2%), in factor 2 were PeCB-118 (13.9%) and PeCB-126 (33.2%), and factor 3 were TeCB-77 (90.9%). The result of PMF of PCNs was showed in Fig 5(c), PCNs were divided into 3 Factors as secondary copper smelter (32.7%) and copper sludge smelter (34.2%) and MWI (33.2%). The main congener in factor 1 was HexaCN (82.0%), in factor 2 was MonoCN and factor 3 was HeptaCN.

Acknowledgements:
The authors gratefully acknowledge the financial support provided by the Environmental Protection Administration, Executive Yuan (EPA110F020).

References:
**MORNING BREAKOUT SESSIONS**

**THURSDAY 14 SEPTEMBER 2023**

**10:00 - 12:00**

**Fate and Transport**

*H. Fiedler & C. Sandau*

**THU-AM-C2**  The Comparison Of Atmospheric Pcdd/Fs, Pcns And Pcbs Between Gas-And Coal-Fired Power Plant In Taiwan

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**Figure 1:** Map of ambient air sampling sites in gas- and coal-fired power plants vicinity in Taiwan.

**Figure 2:** Congener profile of (a) PCDD/Fs, (b) PCBs and (c) PCNs in PM$_{2.5}$ in coal-fired sampling site.

**Figure 3:** The distribution of water-soluble ion in coal-fired power plant in vicinity.

Winter  
(18.5±7.80 µg/m$^2$, n=14)

Summer  
(11.8±2.12 µg/m$^2$, n=14)
THU-AM-C2  The Comparison Of Atmospheric Pcd/Fs, PcnS And PcbS Between Gas-And Coal-Fired Power Plant In Taiwan

Figure 4: The distribution of water-soluble ions in gas-fired power plant in vicinity.

Figure 5: The result of PMF in (a) PCDD/Fs, (b) PCBs and (c) PCNs in coal-fired power plant vicinity.
1. Introduction:
Organochlorine pesticides (OCPs) are persistent organic pollutants (POPs) banned in most or all countries, but continue to cycle in the Earth system. The atmospheric OCP concentrations may be sustained by secondary emissions from oceans and land surfaces (Lammel & Stemmler, 2012), which are only slowly decreasing in the global marine environment (Lohmann et al., 2007), or might even re-emerge in the deep sea (Stemmler & Lammel, 2013). Unlike for northern hemisphere continents and the Arctic, there is no systematic chemical monitoring of the oceans in place and few observational data are available only. Specifically, for DDT since 1990 there are no measurements in open Atlantic seawater and not at all for endosulfan. The direction of air-sea diffusive gas exchange of anthropogenic chemicals has been depositional historically and over many years, but may turn into volatilisational, driven by surface water pollution or by declining atmospheric levels.

2. Materials and Methods:
We determined concentrations in air (gaseous and particulate phases) and surface seawater (dissolved fraction only) along two north-to-south and one east-to-west transect in the Equatorial Atlantic (EA) and South Atlantic (SA) ocean, respectively, as well as in the Mediterranean Sea (MS), Red Sea (RS), Arabian Sea (AS) and Oman and Persian Gulfs (OPG; Table 1).

Sampling of surface seawater was done by dynamic passive sampling (DPS; Sobotka et al., 2021). The DPS device consists of an electrically driven large volume water pump coupled to a passive sampler exposure cell (silicone sheets) placed in a barrel fed with continuously sampled seawater. The equivalent volume of sampled water was estimated from dissipation of performance reference compounds during exposure (Rusina et al., 2010). Air was sampled from an upper deck using a high-volume air sampler (Digitel DH77) equipped with quartz fibre filter (QFF) and 2 polyurethane foam (PUF) plugs. Air and water sampling duration was typically 1 and 2-4 days, respectively. No sampling was only performed outside coastal waters and when the ship was moving. Air sampling periods with influence of the ship's stack in the RS, AS and OPG (Wietzoreck et al., 2022) did not impact on the targeted substances.

In total 13 species (isomers) and, additionally 8 environmental metabolites (isomers) of polychlorinated benzenes (HCB, PeCB), hexachlorocyclohexane (HCH), cyclodienes (endosulfan, DDT, chlordane, aldrin, heptachlor) were targeted. The substances were analysed in the CH<sub>3</sub>CN or CH<sub>2</sub>Cl<sub>2</sub> (Soxhlet) extracts of the silicone, QFF and PUF samples by GC/MS-MS (Waters Xevo TQ-S MS APGC coupled to Agilent 7890 GC and Waters AutoSpec Premier HRMS coupled to Agilent 7890GC). Endosulfan was also analysed using a Q Exactive coupled to a Q Exactive Orbitrap Mass Spectrometer (ThermoFisher). The reported concentrations are blank corrected using the average of three field blanks but not recovery-corrected.
THU-AM-C3  Endosulfan, DDT and other organochlorine pesticides in the Atlantic and Middle East Seas atmosphere, surface waters and air-sea gas exchange

3. Results:
3.1 Concentrations in air and surface waters
Chlorobenzenes, heptachlor, chlordane, DDT compounds, and endosulfan were quantifiable in most of the air and water samples. The pollution levels were low or very low (open ocean background; Table 2, Figure 1).

Table 2: Concentrations in air and surface water (median (min-max)). \( \Sigma \text{DDX} = p,p'-,o,p'-\text{DDT} + -\text{DDE} + -\text{DDD}, \Sigma \text{endosulfan} = \alpha,\beta-\text{endosulfan} + \text{endosulfan sulfate}, \Sigma \text{HCH} = \alpha-,\beta-,\gamma-\text{HCH}, \Sigma \text{heptachlor} = \text{heptachlor} + \text{cis-, trans-heptachlor epoxide}, \Sigma \text{chlordane} = \alpha-,\gamma-\text{chlordane} + \text{oxychlordane}. 

<table>
<thead>
<tr>
<th>Pollutant</th>
<th>Equatorial (EA) and South (SA) Atlantic</th>
<th>Mediterranean Sea (MS), Red Sea (RS), Arabian Sea (AS) and Oman and Persian Gulfs (OPG)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Air (pg m(^{-3}))</td>
<td>Water (pg L(^{-1}))</td>
</tr>
<tr>
<td>PeCB</td>
<td>6.4 (4.8-7.3)</td>
<td>2.0 (0.80-3.0)</td>
</tr>
<tr>
<td>HCB</td>
<td>0.55 (0.32-4.89)</td>
<td>3.2 (&lt;0.7-6.8)</td>
</tr>
<tr>
<td>( \Sigma \text{HCH} )</td>
<td>0.65 (0.11-7.50)</td>
<td>1.13 (&lt;0.5-1.37)</td>
</tr>
<tr>
<td>Aldrin</td>
<td>0.03 (&lt;0.08-0.03)</td>
<td>n.d.</td>
</tr>
<tr>
<td>( \Sigma \text{chlordane} )</td>
<td>0.10 (0.013-0.23)</td>
<td>n.d.</td>
</tr>
<tr>
<td>( \Sigma \text{heptachlor} )</td>
<td>0.11 (&lt;0.10-0.27)</td>
<td>1.06 (0.40-2.87)</td>
</tr>
<tr>
<td>( \Sigma \text{DDX} )</td>
<td>0.58 (0.07-2.91)</td>
<td>0.67 (0.24-4.02)</td>
</tr>
<tr>
<td>( \Sigma \text{endosulfan} )</td>
<td>0.16 (0.03-4.45)</td>
<td>0.67 (&lt;1.5-10.2)</td>
</tr>
</tbody>
</table>
3.2 Direction of diffusive air-sea exchange
The direction of air-sea diffusive gas exchange was derived from simultaneous air and surface seawater concentrations using the Whitman two-film model (Bidleman & McConnell, 1995; Lammel et al., 2016). DDT (and DDE) were volatilising from Equatorial and South Atlantic waters, endosulfan in the western parts of these sea regions.

4. Discussion:
HCB in Atlantic surface seawater was somewhat higher than reported 2004 and 2009 (Lohmann et al., 2009, 2012) and even higher in Middle East Seas. HCH in the SA was found below 2008 levels (Xie et al., 2011). The DDT concentration in Atlantic seawater was similar to 1990 and 2015 levels in the North Atlantic (Iwata et al., 1993; Lammel et al., 2017). Endosulfan had previously not been measured in the Atlantic Ocean; the levels found are similar or higher than those reported from the North Pacific Ocean (Zhong et al., 2012).

5. Conclusions:
Long banned pesticides are still cycling in the marine environment. The findings indicate that the spatial distribution of POPs in the Equatorial and South Atlantic Ocean is not only influenced by riverine inputs and ocean currents, but also by atmospheric deposition from continental plumes and coastal contamination.

6. Acknowledgements:
We thank the organisers of the RV Meteor (M124, M152, M157) and Kommandor Iona (AQABA) campaigns and the ship crews for the great support. This work was supported by the Czech Ministry for Education, Youth and Sports through projects RECETOX RI (LM2023069) and ACTRIS RI (LM2023030), and by the Max Planck Society.

7. References:
THU-AM-C3  Endosulfan, DDT and other organochlorine pesticides in the Atlantic and Middle East Seas atmosphere, surface waters and air-sea gas exchange


Fate and Transport
H. Fiedler & C. Sandau

THU-AM-C4  Fate of PFAS From Soil to Crop and From Substrate to Insect Larvae: Relevance for the Safety of Circular Food Systems

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Introduction: The application of residual streams in food and feed production systems can lead to the transfer and/or accumulation of hazardous chemicals[1], which can have implications for food- and feed safety. So far, several studies have reported on the uptake of PFAS by vegetable crops through biosolid-amended soils, but discrepancies between studies exist and more research is needed[2]. Therefore, transfer of PFAS from biosolid-amended soil to vegetable crops was evaluated. In addition, the transfer of PFAS to insect larvae, suitable for food or feed, was investigated. Insect larvae have the potential to be reared on residual streams, while only limited information on PFAS transfer to insect larvae is available[3]. These studies improve understanding the fate of PFAS through food systems.

Materials and Methods: Two plant species, radish and greens ‘Blauwe Groninger’ (greens; a leafy vegetable), were exposed to PFOS, PFOA, PFHxS and PFNA individually and in mixture, through biosolid-amended soils in a closed pot system (final concentrations in soil: 5 ng/g and 100 ng/g). Concentrations of each of the four PFAS were quantified in roots and shoots by LC-MS/MS. Larvae of the lesser mealworm (LMW; Alphitobius diaperinus) and black soldier fly (BSF; Hermetia illucens) were exposed to PFOS, PFOA, PFHxS and PFNA individually and in mixture. BSF were exposed to 6:2 FTS and 6:2 FTOH as well. Larvae were reared on spiked substrate (final concentrations PFOS, PFOA, PFHxS and PFNA: 5 ng/g, 50 ng/g and 500 ng/g in LMW substrate and 1 ng/g, 10 ng/g and 100 ng/g in BSF substrate). PFAS concentrations were quantified in the harvested larvae by LC-MS/MS.

Results: PFAS concentrations in radish roots ranged from 2.2-5.9 ng/g w.w. (exposure concentration 100 ng/g) in the sequence: PFOA > PFHxS > PFNA > PFOS. A similar pattern was observed for greens shoots with concentrations ranging from 0.5 ng/g to 13.4 ng/g w.w. (exposure concentration 100 ng/g). Differences between roots and shoots of the same plant species were observed. PFAS seemed to transfer to the larvae of both insect species, but transfer patterns differed between the four compounds and two insect species. For LMW, the highest concentrations were determined for PFNA and PFOA, followed by PFHxS and PFOS. For BSF, highest concentrations were quantified for PFHxS, followed by PFNA, PFOA and PFOS. Exposure to the precursors 6:2 FTS and 6:2 FTOH resulted in increased concentrations of other PFAS (such as PFPA, PFHxA) in the BSF larvae.

Discussion and Conclusion: The fate of PFAS differed between the plant species and insect species and does not seem to be fully explained by chain length. The order of PFAS uptake does not fully corroborate with previous findings[3], and determining the corresponding explanatory influential factors would be of interest for future research. Results also indicate the relevance to evaluate fate and metabolism of precursors in food systems, which requires further research.

Acknowledgements: Financial support was provided by the Netherlands Ministry of Agriculture, Nature and Food Quality (plant studies, KB-37-002-038) and the European Union (insect studies, SUSINCHAIN project; H2020 grant agreement No 861976). B. van de Kooi, E. Jongedijk, E. de Lange, K. van Zadelhoff and W. Huang (WFSR) are acknowledged for their help with the plant studies.

References:
THU-AM-C5 Integrating compound-specific stable isotope and enantiomer-specific analysis to characterize the isomeric and enantiomeric signatures of hexachlorocyclohexanes (HCHs) in paddy soils

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**Introduction:** Compound-specific stable isotope analysis (CSIA) is a robust technique for characterizing the transformation pathways of environmental pollutants. The application of CSIA to provide evidence of pollutant degradation within the environmental compartments has advanced rapidly (Liu et al., 2022). Enantiomer fraction (EF) is an indicator of the biotransformation of chiral compounds and can reveal the processes involved in the selective degradation of particular enantiomers (Wu et al., 2019). Soil pollution is a global environmental problem that seriously threatens food security and, eventually, human health. Soil with long-term flooding conditions is an optimum environment for naturally attenuating soil residual organochlorine pesticides via reductive dechlorination (Zhu et al., 2019). In this respect, widely used legacy hexachlorocyclohexanes (HCHs) were investigated in paddy field soils. To characterize the occurrence, degradation, and transformation of HCHs in paddy fields, in correspondence with enantiomeric, isotope profiles, and climatic conditions to elucidate the underlying influencing factors responsible for the redistribution and transformation of HCHs. This study synergized the opportunities of studying the behaviors of compound-specific stable isotopes and enantiomers fractions of HCHs in soils under anaerobic conditions.

**Materials and Methods:** In this study, a field study was conducted in three typical rice-growing regions Taihu Plain (TP), Sanjiang Plain (SP), and Hani Terrace (HT) across a 4,000 km transect of China. GC-MS was used for HCHs detection. The enantiomeric detection of α-HCH was performed by a GC-ECD equipped with a BGB-172 chiral capillary column. The stable carbon isotopes of α, β, γ, and δ-HCH were analyzed by GC combustion-isotope ratio mass spectrometer.

**Results:** The ΣHCHs in TP (mean value of 1.44 ng/g) were significantly (p < 0.05) higher than those in HT (mean value of 0.97 ng/g) and SP (mean value of 0.31 ng/g). The isomer profile showed that β-HCH (49.0-67.9% abundance) was the most predominant in all paddy soils and followed by α-, δ-, and γ-HCH isomers. The isomer ratios of α-/β-HCH (all were below 11.8), α-/γ-HCH (92% < 4.64 > 8%), and the enantiomeric fraction of chiral α-HCH (mean value of 0.81) reflected that the HCHs distribution in the investigated paddy soils were affected by the cocktail usage of technical HCHs and lindane application. The preferential depletion of (-)-α-HCH enantiomers (EFs > 0.5) and pronounced δ¹³C fractionation (ranged from -28.22 ± 0.92‰ to -23.63 ± 1.89‰) of α-HCH demonstrated the effective transformation of α-HCH isomer. However, the δ¹³C isotopes of β-HCH and δ-HCH did not show enrichment, which is consistent with their resistance to degradation.

**Discussion and Conclusion:** The present study provided an effective approach incorporating the compound-specific carbon isotope signature and enantioselective fractionation of HCH isomers for elucidating the occurrence, transformation, and degradation of HCHs in paddy soils from three different regions of China. The residue levels of soil HCHs were found to be much higher in TP than in HT and SP. The nonracemic signatures of α-HCH suggested the preferential depletion of (-)-α-HCH, which was significantly influenced by soil temperature and pH. The CSIA analysis showed enrichment in δ¹³C of α-HCH, revealing that the α-HCH was degraded and transformed in the investigated paddy soils. However, no apparent changes were observed in the...
THU-AM-C5 Integrating compound-specific stable isotope and enantiomer-specific analysis to characterize the isomorphic and enantiomeric signatures of hexachlorocyclohexanes (HCHs) in paddy soils

δ^{13}C isotope values of β- and δ-HCH isomers. The two-dimensional approach of combining enantiomer and δ^{13}C fractionation confirmed the active degradation of α-HCH in the studied paddy soils. In addition, environmental factors were found to be essential factors influencing the transport, fate, and degradation of HCHs. In conclusion, this research established a framework for integrating CSIA and enantiomeric analysis to understand the underlying mechanisms responsible for the transport, fate, and degradation of HCH isomers in paddy soils.

Acknowledgments: This work was funded by the Zhejiang Shuren University Start-up Funds (2021R017).

References:
Introduction:
Various kind of chemicals are associated with PM$_{2.5}$ which can cause health impact on the human body. These chemicals include polycyclic aromatic hydrocarbons (PAHs), persistent organic pollutants (POPs), and other compounds. A previous study presented that PAHs are teratogenic, carcinogetic, and can cause damage to the nervous and immune systems (Sulong et al., 2019). In addition, the polychlorinated dibenzo-p-dioxins and furans (PCDD/Fs), polychlorinated biphenyls (PCBs), and polychlorinated naphthalenes (PCNs) are listed as toxic compounds in POPs in the Stockholm Convention. Past studies have shown that exposure to PCDD/Fs, PCBs, and PCNs can lead to chloracne, hepatotoxicity, cancer, or death (Schecter et al., 2016). According to past research (Li et al., 2017), the pollutants were carried by long-range transport and account for 33% in developing countries. These long-range transport pollution may be caused by open burning events in Southeast Asia or dust storms and monsoon from Northeast Asia. In recent years, the long range transport events have caused global concerns due to its adverse effects on visibility, human health and global climate by increasing particulate matter levels and other gaseous pollutants such as CO, SOx, NOx and VOCs. To understand the effects of these chemical compounds on human health and environment, this study assessed the mutagenicity of PAHs, PCDD/Fs, PCBs and PCNs in the total suspended particulate matter (TSP) and PM$_{2.5}$ in different Asian cities. We used receptor models such as principal component analysis (PCA) and positive matrix factorization (PMF) to analyze the contribution and profile of possible sources. The aim of this study was to measure and analyze the effects of hazardous air pollutants in different cities from anthropogenic emission sources in Taiwan, Hanoi, Chiang Mai, Ulaanbaatar, and Beijing, respectively.

Materials and Methods:
From 2019 to 2023, five urban sampling sites (Taipei, Chiang Mai, Hanoi, Ulaanbaatar and Beijing) were selected from different countries (Fig. 1). In this study, the high volume air sampler (Sibata HV-1000R, HV-500R; Digital DHA-80 and Tisch PS-1) were adopted to collect TSP and cascade impactor for PM$_{2.5}$. The high volume air samplers were equipped with quartz fiber filters for collecting particle-bound compounds while polyurethane foam (PUF) plugs were used for retaining vapor-phase PCDD/Fs, and PCDD/Fs, PCNs, and PCBs. Then we used multi-layer acid gel column and activate carbon to conduct for PCDD/Fs, PMF, and PCBs.

After sampling, the filters were conditioned and weighed to measure its mass concentration and used Soxhlet extraction (Acetone/n-Hexane 1:1(v:v) for 8 hours, 100% Toluene for 16 hours) to extract polycyclic aromatic hydrocarbons (PAHs), polychlorinated dibenz-p-dioxins and dibenzofurans (PCDD/Fs), polychlorinated biphenyls (PCBs) and polychlorinated naphthalenes (PCNs). Then we used multi-layer acid gel column and activate carbon to conduct for PCDD/Fs, PMF, PCBs.

Finally, the analysis was performed using GC-MS/MS (Thermo Fisher Scientific Inc., TRACE 1300 GC & TSQ 8000 Evo triple quadrupole system), covering 27 types of PAHs, 17 types of PCDD/Fs, 12 types of PCBs, and 20 types of PCNs. Additionally, the WHO-TEQ index was used to compare the toxicity of these persistent organic pollutants (POPs).

To identify the different possible sources during the sampling period, the principal component analysis (PCA), and positive matrix factorization (PMF) were used. Moreover, to investigate the possible source regions of PM$_{2.5}$, we calculated 2-3 day back trajectories by HYSSPLIT model for PSCF (potential source contribution function).

Results and Discussion:
In this study, we conducted an investigation on atmospheric pollutants in five different Asian cities, including Taipei, Chiang Mai, Hanoi, Ulaanbaatar, and Beijing. During the sampling period, we measured the concentrations of PM$_{10}$, TSP, Σ27 PAHs TEQ$_{BaP}$, and Σ8 PAHs MEQ$_{BaP}$ in these cities. The results showed that the average concentrations of the TSP concentration in Ulaanbaatar was 75.3 μg/m$^3$, respectively, while PM$_{2.5}$ in Chiang Mai, Hanoi, and Taipei were 116.4, 86.3, and 11.1 μg/m$^3$. In addition, the Σ27 TEQ$_{BaP}$ concentrations in these Asian cities were measured 55.3±8.88, 23.0±24.8, 2.81±2.64, and 6.75±8.88 ng/m$^3$, respectively, while the Σ8 MEQ$_{BaP}$ concentrations were 1.74±1.14, 0.37±0.28, 0.65±0.22, and 0.32±0.35 ng/m$^3$, respectively.
These results indicate that there were significant differences of major anthropogenic emission sources in these cities. The distribution of PAH congeners in Ulaanbaatar is predominantly composed of high and medium molecular weight compounds. This pattern is also observed in Chiang Mai and Hanoi. However, in Chiang Mai, the major source is open burning events while the mobile and vehicular emissions in Hanoi. In Taiwan, there is a noticeable prevalence of high and low molecular weight species, which is attributed to pollution from traffic-related activities. In addition, the contribution of pollution sources to these toxic substances in these cities were analyzed via Principal Component Analysis (PCA). The score plot from the PCA results shows significant differences among the four mega cities. In Chiang Mai, Hanoi, and Ulaanbaatar, the major contributors of PAHs were MMW (Ft, AcPy), HMW (IND, BghiP, BbF and BkF), and LMW (Pyr, Ant), respectively. (Fig. 4).

Finally, the study also assessed the cancer risk of $\Sigma_{16} TEQ_{BAP}$ (Fig. 5). The results showed that the cancer risk of $\Sigma_{16} TEQ_{BAP}$ in Ulaanbaatar, Chiang Mai, and Hanoi was higher than the acceptable cancer risk ($1 \times 10^{-6}$ to $1 \times 10^{-4}$), at $8.88 \times 10^{-6}$, $1.03 \times 10^{-5}$, and $9.21 \times 10^{-6}$, respectively. The measurement results indicated that air pollution has become a serious problem in several cities of developing Asian countries, hence, the intensive and continually ambient air measurement was necessary to improve air quality in the near future.

Acknowledgements:
The authors acknowledge the Ministry of Science and Technology of Taiwan (MOST 111-2111-M-A49-001).

Reference
THU-AM-C6  Toxicity And Mutagenic Risk Assessment Of Atmospheric PM Bound PAHs And PCDD/Fs In Different Asia Cities

Fig. 2 The concentration of TSP, PM2.5, Σ27PAH TEQBaP and Σ8PAH MEQBaP in different Asia cities.

Fig. 3 The distribution of Σ27PAH congener in different Asia cities.
THU-AM-C6  Toxicity And Mutagenic Risk Assessment Of Atmospheric PM Bound PAHs And PCDD/Fs In Different Regions

Fig. 4 The result of score plot and congener difference in PCA in different Asia cities.

Fig. 5 The ICR of Σ16PAH TEQBaP and Σ8PAH MEQBaP in different Asia cities.
THU-AM-D1  Screening of Brominated and Chlorinated Additives in Plastic Pellets

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2 POPs Environmental Consulting, Schwäbisch Gmünd 73527, Germany

Introduction: Most of the newly added persistent organic pollutants (POPs), listed for global elimination under the Stockholm Convention, are plastic additives, some are brominated additives, such as polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane, and some are chlorinated additives, such as short-chain chlorinated paraffins and Dechlorane Plus. These plastic additives have been widely used in variety of plastic products including polyurethane foam, electronic casings, textiles, building insulation, etc., usually on the order of % by weight. To promote the recycling of plastics for a circular economy, it is necessary to eliminate plastics containing POPs from the recycling stream. Otherwise, recycled plastics containing POPs inadvertently contribute to the contamination of new plastic products when POPs are not effectively eliminated from recycling streams1. However, individually analyzing each compound through chemical analysis can be time-consuming and expensive. Thus, it is effective to quickly screen the content of bromine (Br) and chlorine (Cl) in plastic products and wastes by X-ray fluorescence (XRF) as indicators of the presence of POPs in plastic. Samples identified as potential candidates for further confirmation can be then selected for subsequent chemical analysis. The aim of this study is to screen POP contents in recycled plastic pellets obtained from developing countries, where information on POPs in circulation is particularly limited.

Materials and Methods: A total of 253 plastic pellets derived from recycled plastic have been collected in Africa (Ghana, Nigeria), Asia (Indonesia, Mongolia, Thailand, Vietnam), and South America (Argentina, Brazil, Chile), between February and April 2023. Additionally, 16 pellet samples collected in 2021 from South America were also included in this study. Following the findings of previous studies2,3, the elemental Br content in pellet samples was determined as a surrogate for brominated flame retardant content. A handheld XRF analyzer (Olympus Vanta C Series) was used for non-destructive analysis, providing an estimate of the average Br content in the scanned area of an item. Before use, the handheld XRF analyzer was calibrated using peak positions of iron and molybdenum, the peak width at half height of manganese, and the total count of SUS316 stainless steel. The screening survey was conducted in RoHS/WEEE mode, with a reading time set at 30 seconds. Components with a Br content ≥15 mg kg−1 by weight were considered Br-positive and selected for further chemical analysis.

Results: Since individual pellets were too small to measure using XRF, the pellets were combined in a thin plastic bag, and the entire bag was repeatedly measured to assess variations in Br content. As a result, the coefficient of variation was sufficiently small, around 6%, even for samples with low Br content, indicating that the plastic pellets could be considered homogeneous. Among the 269 pellet samples, XRF screening revealed that 72% had Br contents <15 mg kg-1, suggesting that these samples were unlikely to contain PBDEs above the low POP content (LPC) proposed under the Basel Convention. Therefore, these samples could be omitted from the detailed chemical analysis for confirmation. The remaining 74 pellet samples (28% of the total) showed Br contents >15 mg kg−1, indicating a potential presence of POPs that should be confirmed through future chemical analysis.

Discussion and Conclusion: XRF screening is a useful tool for pre-selecting samples for detailed POP analysis. Indeed, it facilitated the omission of detailed POP analysis for over 70% of the samples in this study.

Acknowledgments: We acknowledge GEF for funding of the UNEP GMP project and the Environment Research and Technology Development Fund [grant numbers JPMEERF20193001, JPMEERF20233001] of the Environmental Restoration and Conservation Agency provided by Ministry of the Environment of Japan for partially funding this study.

References:
THU-AM-D2  Determination of PCDD/F, PCB and PBDE in different ocean plastic samples collected in Atlantic Ocean

Kay Kelterer1, Marco Fortmann1, Annika Huebner1, Markus Schroeder1, Eckard Jantzen1
1 GALAB Laboratories GmbH, Am Schleusengraben 7, Hamburg 21029, Germany, kay.kelterer@galab.de

1. Introduction:
A huge amount of plastic waste has been released into the marine environment since decades and still is released every year. The ongoing trend for research in the field of ocean plastic brought the opportunity to check these kinds of samples for different halogenated contaminants.

2. Materials and Methods:
All samples for this study were collected at the Atlantic Ocean between 33° - 35° latitude north and 31° - 39° longitude west during expedition POS 536 of the BMBF-founded PLASTISEA project by GEOMAR - Helmholtz Centre for Ocean Research, Kiel. Different plastic samples in size, type and original use were gathered. The used inhouse method covers the 17 isomers of the 2,3,7,8-substituted PCDD/F, 44 PCB (incl. 12 WHO-PCB and 6 indicator PCB), 10 different PBDE and PBB. The samples have been cold extracted using sulphuric acid and n-hexane, after adding the 13C12-labelled internal standards for PCDD/F, the 2,3,7,8-substituted PCDD/F, 44 PCB (incl. 12 WHO-PCB and 6 indicator PCB), 10 different PBDE and PBB. The extract was treated with sodium sulphate and copper powder. Afterward a cleanup via the automated necessary evaporations have been done by a SuperVap from FMS Inc or a gentle stream of nitrogen. After evaporation, before injection, a recovery standard using four 13C12-labelled PCDF and 13C12-labelled PCB has been added. The measurement of the final extracts, one for PCDD/F incl. 4 coplanar PCB and one for the remaining PCB, PBDE and PBB was performed by Agilent 7010B triple quadrupole GC/MS. For the measurement of PCDD/F a VF-Xms column from Agilent, for the PCB the HT8 from Trajan and for the PBDE and PBB the DB-XLB from Agilent were used. All deployed standard solutions (native and 13C12-labelled) have been prepared by different ready to use mixtures.

3. Results:
The analytical results are shown in Table 1 to Table 3. All positive results above the reporting limit are shown in bold, analytes detected but below the reporting limits are marked with "< reporting limit" with the calculated amount of the detected signals in brackets. Analytes below the detection limit of the method are listed with "n.d." in brackets for not detected.

Table 1: Results for PBDE and PBB

<table>
<thead>
<tr>
<th>sample ID</th>
<th>5</th>
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<th>41</th>
<th>60</th>
<th>61</th>
<th>62</th>
<th>63</th>
<th>64</th>
<th>125</th>
<th>G1</th>
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<tr>
<td>(µg/kg)</td>
<td></td>
<td></td>
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<tr>
<td>PBDE #28</td>
<td>&lt;0.012</td>
<td>&lt;0.045</td>
<td>&lt;0.218</td>
<td>&lt;0.027</td>
<td>&lt;0.028</td>
<td>&lt;0.021</td>
<td>&lt;0.023</td>
<td>&lt;0.039</td>
<td>&lt;0.261</td>
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<tr>
<td></td>
<td>(n.d.)</td>
<td>(0.025)</td>
<td>(n.d.)</td>
<td>(0.006)</td>
<td>(n.d.)</td>
<td>(0.013)</td>
<td>(n.d.)</td>
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<td>&lt;0.039</td>
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<tr>
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<td>0.049</td>
<td>&lt;0.218</td>
<td>&lt;0.027</td>
<td>&lt;0.028</td>
<td>&lt;0.021</td>
<td>&lt;0.023</td>
<td>&lt;0.039</td>
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<td>&lt;0.028</td>
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<td>&lt;0.039</td>
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<td>&lt;0.027</td>
<td>&lt;0.028</td>
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<td></td>
<td>(n.d.)</td>
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<td>&lt;0.871</td>
<td>&lt;0.109</td>
<td>&lt;0.110</td>
<td>&lt;0.111</td>
<td>&lt;0.083</td>
<td>&lt;0.092</td>
<td>&lt;0.144</td>
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<td>PBDE #153</td>
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<td>&lt;0.182</td>
<td>&lt;0.871</td>
<td>&lt;0.109</td>
<td>&lt;0.110</td>
<td>&lt;0.111</td>
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<td>PBDE #154</td>
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<td>&lt;0.182</td>
<td>&lt;0.871</td>
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<td>&lt;0.110</td>
<td>&lt;0.111</td>
<td>&lt;0.083</td>
<td>&lt;0.092</td>
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<td>PBDE #183</td>
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<td>&lt;0.138</td>
<td>&lt;0.139</td>
<td>&lt;0.103</td>
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Table 2: Results for PCDD/F

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<th>61</th>
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<th>64</th>
<th>125</th>
<th>G1</th>
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<td>µg/kg</td>
<td>µg/kg</td>
<td>µg/kg</td>
<td>µg/kg</td>
<td>µg/kg</td>
<td>µg/kg</td>
<td>µg/kg</td>
<td>µg/kg</td>
<td>µg/kg</td>
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<tr>
<td>PBB #138</td>
<td>&lt;0.031 (n.d.)</td>
<td>&lt;0.184 (n.d.)</td>
<td>&lt;0.544 (n.d.)</td>
<td>&lt;0.569 (n.d.)</td>
<td>&lt;0.070 (n.d.)</td>
<td>&lt;0.052 (n.d.)</td>
<td>&lt;0.058 (n.d.)</td>
<td>&lt;0.099 (n.d.)</td>
<td>&lt;0.653 (n.d.)</td>
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<td>OCDF</td>
<td>1.70</td>
<td>2.176 (0.270)</td>
<td>0.817 (0.270)</td>
<td>0.719 (0.270)</td>
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<td>&lt;2.611 (n.d.)</td>
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<tr>
<td>OCDD</td>
<td>61.2</td>
<td>7.05</td>
<td>&lt;2.176 (0.810)</td>
<td>0.817 (0.270)</td>
<td>0.719 (0.270)</td>
<td>0.755 (0.270)</td>
<td>25.7</td>
<td>2.30</td>
<td>&lt;2.611 (n.d.)</td>
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THU-AM-D2  Determination of PCDD/F, PCB and PBDE in different ocean plastic samples collected in Atlantic Ocean
MORNING BREAKOUT SESSIONS

THURSDAY 14 SEPTEMBER 2023

10:00 - 12:00
POPs and Microplastics
M.Elskens & S.Harrad
THU-AM-D2	Determination of PCDD/F, PCB and PBDE in different ocean plastic samples collected in
Atlantic Ocean
Table 3: Results for PCB
sample ID

5

17

41

60

61

62

63

64

125

G1

analyte

ng/kg

ng/kg

ng/kg

ng/kg

ng/kg

ng/kg

ng/kg

ng/kg

ng/kg

ng/kg

PCB #18

21.5

282

<54.4 (15.7)

16.4

26.0

44.0

10.7

36.0

18.6

<65.3 (17.7)
<65.3 (n.d.)

PCB #28

13.6

291

<54.4 (n.d.)

<6.82 (4.80) <6.90 (1.97) <6.95 (2.23) <5.17 (0.87) 16.4

10.0

PCB #44

11.0

896

<54.4 (n.d.)

7.77

<6.90 (6.00) <6.95 (6.58) <5.17 (4.49) 14.5

<9.87 (9.28) <65.3 (n.d.)

PCB #49

7.44

827

<54.4 (7.13)

15.6

13.2

15.7

11.6

8.43

<9.87 (5.90) <65.3 (n.d.)

PCB #52

17.5

1666

<54.4 (14.9)

18.6

16.2

18.1

12.5

22.2

12.5

<65.3 (16.9)

PCB #66

4.89

1281

<54.4 (n.d.)

<6.82 (3.85) <6.90 (2.51) <6.95 (2.68) <5.17 (2.06) <5.77 (3.47) <9.87 (2.72) <65.3 (n.d.)

PCB #74

3.29

721

<54.4 (n.d.)

<6.82 (1.94) <6.90 (1.16) <6.95 (1.33) <5.17 (0.87) <5.77 (1.90) <9.87 (1.73) <65.3 (n.d.)

PCB #77

<1.23 (0.517)

727

<21.8 (1.36)

<2.73 (1.52) <2.76 (1.45) <2.78 (1.65) <2.07 (1.27) <2.31
(0.603)

3.96

<26.1 (n.d.)

PCB #81

<0.031 (0.016)

28.5

<0.544 (n.d.)

<0.068
(0.048)

<0.099
(n.d.)

<0.653
(n.d.)

PCB #87

<3.08 (1.97)

2633

<54.4 (n.d.)

<6.82 (3.93) <6.90 (2.67) <6.95 (3.32) <5.17 (1.82) <5.77 (1.28) <9.87 (1.35) <65.3 (n.d.)

PCB #95

6.89

2516

<54.4 (10.5)

15.6

<0.069
(0.056)
18.5

<0.200
(0.086)
15.8

<0.200
(0.077)
10.6

<0.058
(n.d.)
8.37

<9.87 (8.22) <65.3 (10.4)

PCB #99

<3.08 (1.14)

2415

<54.4 (n.d.)

21.3

17.5

28.6

18.4

<5.77 (1.34) <9.87 (2.60) <65.3 (n.d.)

PCB #101

4.74

6050

<54.4 (3.96)

62.3

50.8

78.4

49.1

<5.77 (4.41) <9.87 (7.65) <65.3 (n.d.)

PCB #105

<3.08 (0.82)

2423

<54.4 (n.d.)


PCB #110

3.42

5365

<54.4 (n.d.)

15.5

12.9

17.0

11.8

<5.77 (3.53) <9.87 (3.65) <65.3 (n.d.)

PCB #114

<3.08 (n.d.)

136

<54.4 (n.d.)


PCB #118

<3.08 (2.67)

6173

<54.4 (n.d.)

<6.82 (n.d.) <6.90 (n.d.) <6.95 (n.d.) <5.17 (n.d.) <5.77 (1.84) <9.87 (2.51) <65.3 (n.d.)

PCB #123

<3.08 (n.d.)

63.6

<54.4 (n.d.)


PCB #126

<0.031 (0.024)

111

<0.544 (n.d.)

<0.200
(0.090)

PCB #128

<3.08 (0.46)

1810

<54.4 (n.d.)


<0.200
(0.138)

<0.300
(0.218)

<0.200
(0.106)

<0.200
(0.089)

<0.300
(0.219)

<0.653
(n.d.)

PCB #138

4.36

6647

<54.4 (n.d.)

<6.82 (n.d.) <6.90 (n.d.) <6.95 (n.d.) <5.17 (n.d.) <5.77 (4.45) <9.87 (5.06) <65.3 (n.d.)

PCB #146

<3.08 (0.48)

962

<54.4 (n.d.)

48.5

PCB #149

<3.08 (2.30)

3269

<54.4 (3.72)

<6.82 (5.76) <6.90 (4.05) <6.95 (6.46) <5.17 (4.31) <5.77 (1.94) <9.87 (4.05) <65.3 (n.d.)

PCB #151

<3.08 (0.91)

733

<54.4 (2.84)

<6.82 (n.d.) <6.90 (n.d.) <6.95 (n.d.) <5.17 (n.d.) <5.77 (0.90) <9.87 (n.d.) <65.3 (n.d.)

38.0

22.4

71.0

38.7

42.8

27.0

<5.77 (1.09) <9.87 (2.57) <65.3 (n.d.)

PCB #153

3.70

6713

<54.4 (n.d.)

28.6

PCB #156

<3.08 (0.32)

623

<54.4 (n.d.)


<5.77 (5.31) 10.1

PCB #157

<3.08 (n.d.)

161

<54.4 (n.d.)


PCB #158

<3.08 (0.61)

950

<54.4 (n.d.)


<65.3 (n.d.)

PCB #167

<3.08 (n.d.)

349

<54.4 (n.d.)


PCB #169

<0.031 (n.d.)

2.47

<0.544 (n.d.)

<0.068
(n.d.)

<0.069
(n.d.)

<0.069
(n.d.)

<0.052
(n.d.)

<0.058
(n.d.)

<0.099
(n.d.)

<0.653
(n.d.)

PCB #170

<3.08 (n.d.)

657

<54.4 (n.d.)


PCB #172

<3.08 (n.d.)

132

<54.4 (n.d.)


PCB #177

<3.08 (n.d.)

340

<54.4 (n.d.)


PCB #178

<3.08 (n.d.)

119

<54.4 (n.d.)


PCB #180

<3.08 (1.49)

1463

<54.4 (n.d.)

<6.82 (3.01) <6.90 (n.d.) <6.95 (n.d.) <5.17 (1.63) <5.77 (3.03) <9.87 (n.d.) <65.3 (n.d.)

D IOX IN 20 23 - BO O K O F ABST RACTS

386


THU-AM-D2  Determination of PCDD/F, PCB and PBDE in different ocean plastic samples collected in Atlantic Ocean

<table>
<thead>
<tr>
<th>sample ID</th>
<th>5</th>
<th>17</th>
<th>41</th>
<th>60</th>
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<td>analyte</td>
<td>ng/kg</td>
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<td>&lt;6.82 (n.d.)</td>
<td>&lt;6.90 (n.d.)</td>
<td>&lt;6.95 (n.d.)</td>
<td>&lt;5.17 (n.d.)</td>
<td>&lt;5.77 (n.d.)</td>
<td>&lt;9.87 (n.d.)</td>
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<td>&lt;54.4 (8.69)</td>
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<td>&lt;6.90 (n.d.)</td>
<td>&lt;6.95 (n.d.)</td>
<td>&lt;5.17 (1.49)</td>
<td>&lt;5.77 (2.51)</td>
<td>&lt;9.87 (n.d.)</td>
<td>&lt;65.3 (n.d.)</td>
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4. Discussion:
Over all analytes sample 17 was the only sample that showed contamination for all parameters. The highest absolute concentrations for PCB (6713 ng/kg for PCB #153) and PBDE (0.428 µg/kg for PBDE #47) were detected in sample 17. Especially due to the concentration and distribution of the determined PCB in sample 17, it can be suspected that sample 17 had direct contact to different AROCLOR mixtures during its lifetime. The detected and quantified PCB concentrations on the other analyzed samples also seem to be created by different AROCLOR mixture, but not through direct contact, as the concentrations are much lower than for sample 17. The highest concentration for PCDD/F (61.2 ng/kg for OCDD) was found on sample 5. During other studies of these samples for sample 5 and 64, pentachlorophenol (PCP) was detected. It is expected that the contamination of OCDD and 1234678-HpCDD of sample 5 and 64 is caused by the contamination of PCP in these two samples.

5. Conclusions:
In all samples PCB were detected and, in some samples, PCDD/F and PBDE have been detected. The exact source especially of the PCB contamination cannot be defined, but it can be estimated, that the contaminations are caused by ab- and/or adsorption processes during the use and/or the floating in the sea of the plastic particles.

6. Acknowledgments:
Erik Borchert and the GEOMAR - Helmholtz Centre for Ocean Research for collecting and providing the samples. Frank Neugebauer (GfA Lab Service) for fruitful discussion on PCB patterns.

7. References:
Micro(nano)plastics in the atmosphere of the Atlantic Ocean

Marinella Farré²,* Elisa Caracci¹,a, Albert Vega-Herrera²,a, Jordi Dachs², Naiara Berrojalbiz², Giorgio Buonanno¹,³, Esteban Abad², Marta Llorca², Teresa Moreno³,*

¹ Department of Civil and Mechanical Engineering, University of Cassino and Southern Lazio, FR, Cassino, Italy
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Introduction: The ubiquitous occurrence of plastics in the marine environment has raised concerns about their persistence and impact on organisms. Plastics have the potential to be transported globally by oceanic currents, a key transport pattern explaining the accumulation of plastics in the centre or convergence region of the subtropical oceanic gyres [1]. The biogeochemistry of plastics in the water column will affect this oceanic transport. However, in recent years, the atmospheric transport and occurrence of plastics have been suggested as key to understanding the long-range transport of microplastics (MPs) (1 µm to 5 mm), and nanoplastics (NPLs) with sizes smaller than 1 µm. Whereas the transport of soot carbon, tyre residues, nanoparticles and other anthropogenic particles have been studied in the past, in contrast, there are large uncertainties on the long-range transport of MNPLs over the oceans. Here we show the characterisation of MNPLs and the aerosol composition (PM10) in a north-south Atlantic transect from Vigo (Spain) to Punta Arenas (Chile).

Materials and Methods: The analytical to assess the composition of MNPLs consisted of a double suspect screening approach of the polymers and additives of plastics. Polymers were analysed by size exclusion chromatography coupled with high-resolution mass spectrometry using an atmospheric pressure photoionization source operated in positive and negative conditions (HPLC(SEC)-APPI(+/-)-HRMS). Plastic additives were screened with high-performance liquid chromatography coupled to high-resolution mass spectrometry using an electrospray ionisation source (HPLC-ESI(+/-)-HRMS). Moreover, this information was complemented by the characterisation of the largest particles using scanning electron microscopy (SEM) and µ-Fourier Transform Infrared Spectroscopy (µ-FTIR).

Results: The most common polymers were polyethylene (PE), polypropylene (PP), polyisoprene (PI), and polystyrene (PS), with the highest polymer concentration being 51.7 ng·m⁻³ of PI. The air mass back trajectories showed the variable influence of oceanic and terrestrial air masses. These differences were reflected in the aerosol composition with different contributions of Saharan dust, sea spray aerosol, organic/elemental carbon, and MNPLs. Results showed that samples largely influenced by sea-spray and air masses originating from coastal South America and the north Atlantic subtropical gyre were more contaminated by MNPLs. This work provides the first field evidence of the long-range transport of MNPLs in most of the Atlantic Ocean, as the result of dynamic coupling between the lower atmosphere and the surface ocean.

Discussion and Conclusion: PE and PI were the most quantified polymers, together with PDMS, which was tentatively identified in 90 % of the samples but not quantified. In general, the most contaminated samples are those influenced by south American coastal airflows and where sea-spray aerosol has a substantial contribution, considering the backward trajectories for each sampling transect. This is the first research work focused on the impact of MNPLs in airborne particulate matter with at least one dimension inferior in size to 10 µm from a north-south Atlantic Ocean transect covering a large oceanic region from the two hemispheres. The approach has allowed determining their occurrence and opened the path for future studies that require quantitative determination of MNPLs for the elucidation of the processes affecting their fate and transport.

Acknowledgments: This work was supported by IMAGE (ref. PID2020-116789RB-C41) and ANTOM (PGC2018-096612-B-100) research projects from the Spanish Ministry of Science and Innovation. Albert Vega-Herrera gives thanks for his grant PRE2018-083989 from the Spanish Ministry of Science and Innovation. The authors also thank the crew on board R/V “Sarmiento de Gamboa” and technicians from UTM-CSIC for their technical support during the sampling campaign. IDAEA-CSIC is a Severo Ochoa Centre of Research Excellence (Spanish Ministry of Science and Innovation; Project CEX2018-000794-S).

References:
Vertical distribution of microplastics in agricultural soil

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Introduction: Our growing population relies on highly productive soil to provide ecosystem services critical to effective agroecosystems. Plastic materials are used to improve soil productivity by reducing evaporation, stabilizing soil temperature, and delivering nutrients. However, the use of plastic and their unintentional introduction through biosolids and irrigation water has led to widespread plastic and microplastic (< 1mm, MP) accumulation in soil rendering it a known sink for plastic¹. Despite previously reported adverse impacts of MPs on soils’ ecosystem functions, including nutrient cycling and water regulation² only a limited number of studies have quantified MP concentrations in soil, particularly outside of China³. In general, MP concentrations and distribution in the subsoil is largely unknown despite laboratory evidence of vertical MP migration in soil⁴. The few studies investigating the MP distribution in soil relied on visual particle identification and spectroscopy measurements, potentially leading to an underestimation of the concentration of the smallest particles⁵,⁶.

This knowledge gap is significant as the vertical migration of MPs has the potential to contaminate underlying aquifers posing a risk to drinking water supplies. To address this, our study will quantify the vertical distribution of MPs in various soil types and agricultural uses, in order to better understand the risk of MP contamination in groundwater systems.

Materials and Methods: Undisturbed soil profile samples (~1.2 m depth) from three different soil types and land uses were taken in Germany (sandy soil – biosolid storage, loess soil – compost, clay soil – plastic mulch) and cut in different length (10-50 cm) with increasing resolution towards the topsoil layer. The samples were oven dried at 50°C, yielding sample weights ranging from 0.55 – 0.85 kg, and subsequently fractionated using stainless steel mesh sieves (1000 μm, 250 μm, 25 μm). After, 10 g of sample from each fraction was weight out into precleaned accelerated solvent extraction (ASE) cells. Samples were spiked with 40 μg of polystyrene (PS-d5) internal standards and ASE extracted using DCM at 180 °C and 1500 psi⁷. 80 – 240 μl of each sample extract was transferred into pyrolysis cups, evaporated and loaded on the Pyr-GC/MS auto sampler for analysis. Field and processing blanks were processed alongside the samples. Sevan plastics (PS, PC, PMMA, PP, PET, PE, and PVC) were analyzed by a double-shot Pyr-GC/MS method as previously reported⁷.

Results: Fractionated samples are currently being analyzed for their MP concentrations. Results are expected to show the highest plastic concentration occurring in the top 30 cm soil layer (plowing layer) as well as the highest concentration of larger (above 250 μm particles). A decrease of microplastic concentration along the soil profile is expected with an increase of smaller particle sizes.

Discussion and Conclusion: This study will be one of the first to present vertical MP concentrations in various soil and land use types enabling a better understanding for the risk of groundwater contamination by MPs. The Pyr-GC/MS analytical method quantifies MPs likely to be missed by spectroscopic and visual methods reducing the uncertainty of MP concentration measurements in soil.

Acknowledgments: This project has received funding from the European Union’s Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 101033462. The authors like to thank the farmers for side access and ongoing collaboration.

References:
POPs and Microplastics

M.Elskens & S.Harrad

THU-AM-D5  Analysis of Additives Leaching from Plastics as a Result of Sludge Disintegration

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Introduction: Microplastics (MPs) are plastics with dimensions less than 5 mm. Wastewater treatment plants have a significant place in the migration of MPs into the environment. Over 90% of the MPs reaching WWTPs are removed and accumulated in sludge that is used later in anaerobic digestion and land application. Plastics contain additives that are mostly not chemically bound to the polymer and are susceptible to leaching (Wiesinger et al., 2021). Various pretreatment methods are applied before anaerobic digestion to enhance the process. These processes are expected to cause an increase in the leaching of these additives from MPs and put the land application of sludge at risk. This study aims to determine whether disintegration poses a stress factor on MPs and leads to the leaching of plastic additives in sludge.

Materials and Methods: Polyethylene Terephthalate (PET), polypropylene (PP), and polycarbonate (PC) plastics between 250-500 µm were used as MP sources. Five different pretreatments were applied to the MPs containing sludge samples taken from a municipal WWTP in Ankara, Turkey. In alkali disintegration, 0.5 M NaOH was added to each sample, and incubated at 25°C for 2 days. Thermal disintegration was applied at 127°C for 2 hours in an autoclave. In enzymatic pretreatment, 200 mg/g TS pancreatin enzyme was used for each sample treatment. The combinations of alkali-thermal and thermal-enzyme pretreatments were also conducted. Then all samples were centrifuged at 4000 rpm for 5 minutes. Prior to filtration, pH was adjusted to 3.0 with HCl. The solid-phase extraction (SPE) cartridges were conditioned, the sample was passed through and then pretreatments were also conducted. Then all samples were centrifuged at 4000 rpm for 5 minutes. Prior to filtration, pH was adjusted to 3.0 with HCl. The solid-phase extraction (SPE) cartridges were conditioned, the sample was passed through and then analytes were eluted with hexane, methanol, and acetoniitrile, sequentially. Quantification was done using gas chromatography coupled with mass spectrometry (GC-MS). Plastic additives analyzed according to their abundance in target plastics were diethyl phthalate (DEP), dibutyl phthalate (DBP), di(2-ethyl hexyl phthalate) (DEHP) and bisphenol-A (BPA).

Results: Alkali treatment caused an increase in DEP concentration and minor increases in DEHP in the liquid phase, whereas thermal disintegration significantly increased DEHP concentration leaching from PC while causing minor DEP concentration increases in both PET and PC MPs. Enzymatic disintegration showed some minor effects similar to alkali treatment where DEP increased in PET and PC, but no other significant changes were observed for other additives. In thermal-enzyme combined disintegration, sludge with PC MPs showed increased BPA levels and minor increases in DEHP for PP and PC MPs. Lastly, the greatest effect was demonstrated with alkali-thermal disintegration where BPA levels in PC-added sludge samples showed a drastic change from around 25 ppb up to 3.5 ppm.

Discussion and Conclusion: An effective SPE method was established for the extraction of possible additives that may leach from MPs. In alkali disintegration, the most noticeable changes have been observed in PET in which DEP concentrations increased significantly. This can be explained by the hydrolysis of PET due to the interaction with NaOH due to its ester bonds (Li et al., 2020). Alternatively, in thermal disintegration, the most noticeable difference was observed on PET. Because of having ester functional groups in the polymeric backbones of PC, it can be easier to depolymerize PC as found in the literature (Li et al., 2022); therefore, leaching of additives from PC can be expected. However, there was only a minor change due to enzymatic pretreatment and this can be confirmed by the literature (Lusher et al., 2017) where enzymatic treatment is used to recover MPs from biological matrices and considered non-damaging for MPs. Combined disintegration methods proved to be the most effective methods and alkali-thermal treatment showed the leaching of BPA from PC plastics as expected since BPA is the monomer of PC. Results show that MPs can leach out plastic additives during sludge disintegration methods and these can be reliably quantified by SPE followed by GC-MS.

Acknowledgments: This study was funded by TUBITAK Project No: 121Y156.

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Introduction: Brominated flame retardants (BFRs) e.g. polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCDD), have been widely used as additives in different type of polymers to impact specific functions despite their well-known toxicities. Since BFRs are additively added to plastics, they can potentially leach out of the matrix, hence microplastics (MPs) are thought to be a driver for BFRs in the environment because of their large exterior surface area. Current understanding is that human exposure to MPs occurs through a combination of ingestion, inhalation and dermal contact due to the presence of MPs in indoor dust, consumer products, water, foodstuff and air. While there is little evidence of adverse health effects associated directly with exposure to pristine MPs, there is major concern over potential toxicity from released plastic additives e.g. BFRs, many of which have exhibited endocrine disruption, neurotoxicity, hepatotoxicity and other toxic effects. While few recent studies reported preliminary assessments of human exposure to MPs via different pathways, there exists no data on the dermal uptake of additive chemicals e.g. BFRs through contact with MPs. In the current study, dermal absorption of BFRs from different types of polymers was experimentally assessed for the first time using in vitro 3D-human skin equivalents (EpiSkin™) under different real-life exposure scenarios.

Materials and Methods: The dermal exposure experiments were performed according to a previously published protocol (Abdallah & Harrad, 2022). Briefly, 3D-HSE skin tissues (1.07 cm² /tissue) were mounted in standard diffusion cells with the stratum corneum facing up. Each tissue was initially equilibrated with the maintenance medium for 30 min at 37 °C before the MPs (50 mg/cm²) was applied onto the skin surface in the donor compartment. No further pressure or weight was applied on top of the MP to avoid potential tearing or loss of the 3D-HSE tissue integrity. To study the influence of skin hydration on dermal uptake of target BFRs, the skin surface was “moistened” with 50 µL/cm² of skin surface film liquid (SSFL), reflecting a “sweaty skin scenario”; while 10 µL/cm² was added in the respective “dry contact” experiments to reflect more “dry skin scenario”. All experiments were performed in triplicate. A DMEM-based culture medium was used as receptor fluid, maintained at 37 ± 1 °C throughout the exposure experiment (24 h). At fixed time points, aliquots of the receptor fluid (2 mL) were collected from the receptor compartment (4 mL capacity) and immediately replaced with fresh fluid. After 24 h, the MPs was removed, the entire receptor fluid was collected and the skin surface washed thoroughly with cotton buds impregnated in (1:1) hexane:ethyl acetate (5 times) to "wipe out" any unabsorbed BFR on the skin surface. The skin tissues were removed from the permeation devices and both the donor and receptor compartments were washed separately (5 times x 2 mL) with (1:1 v/v) hexane:ethyl acetate. All samples were stored at − 20°C until chemical analysis of BFRs. Chemical analysis for target BFRs followed our previously established method (Abdallah et al. 2017).

Results: Results showed that majority of target BFRs were dermally bioavailable to varying degrees following 24 hr skin contact with MPs. Permeation coefficients (Kp, cm h⁻¹), lag time (h) and steady state flux (Jss, ng/cm²·h) were established for these chemicals.

Discussion and Conclusion: The results revealed that dermal uptake of BFRs through contact with MPs depended on the polymer type and specific physicochemical properties of the chemical. Exposure assessment showed that children and adults are exposed to varying levels of BFRs through dermal contact with MPs, hence elucidating the significance of the dermal pathway as a route of human exposure to additive chemicals in MPs.

Acknowledgment: The research leading to these results has received funding from the European Commission’s Horizon 2020 under the Marie Sklodowska - Curie Individual Fellowship (Grant Agreement Number: 101026229).

1. Introduction:
The Panel on Contaminants in the Food Chain of the European Food Safety Authority (EFSA) published a risk assessment on PFASs in 2020 [1]. In the assessment, a tolerable weekly intake (TWI) of 4.4 ng/kg bw per week was established for the sum of PFOA, PFNA, PFHxS and PFOS (ΣPFAS4). The TWI was derived from a human study, in which a lowest BMDL10 of 17.5 ng/ml in serum was identified for 1-year-old children. Using PBPK modelling, this serum level of 17.5 ng/ml in children was estimated to correspond to long-term maternal exposure of 0.63 ng/kg bw per day. The TWI should prevent mothers from reaching a body burden that would result in levels in milk that would in turn lead to serum levels in the infant that are associated with harmful effects on the immune system.

The CONTAM Panel concluded that parts of the European population exceed this TWI, which is of concern. In Finland, ΣPFAS4 intake is estimated to exceed the TWI of 4.4 ng/kg bw per week. However, the health implications of this are uncertain, since presumably, the serum levels of children do not exceed the serum level of 17.5 ng/ml, which was used as the basis for the derivation of the TWI.

Thus, precipitous interpretations of TWI exceedances might lead to risk management measures that will inadvertently impair the overall health of the Finnish population. Risk management decisions should preferably be based on true biomonitoring results, when those are available, with as little uncertainties introduced from computer modelling as possible.

Currently, a biomonitoring study is ongoing in Finland, where serum PFAS levels of 300 1-year-old children are being analyzed. The goal of the study is to investigate, in the light of the new EFSA risk assessment, whether PFASs are a relevant risk to the immune system of Finnish children and to evaluate how this possible risk should be managed. The samples are currently being analyzed in the laboratory and results will be available by July 2023 and presented in the Dioxin 2023 conference.

2. Materials and Methods:
2.2 Serum samples
For this study, serum samples of 50 male and 50 female children born in 2015–2018 from Turku, Tampere and Oulu cities (altogether 300 samples) are available. The samples for this study will be collected from an existing sample bank (DIPP study) [2]. The study has been reviewed and approved by an ethical committee, and the approval includes the use of samples for PFAS analysis.

2.3 Chemical analysis
The analytes are listed in Table 1. The sample volume used for PFAS analysis is 0.1 ml.

The analytical procedure for measurement of PFAS from the serum samples has been described previously [3]. In short, the procedure consists of PFAS extraction and protein precipitation with methanolic ammonium acetate, and instrumental
PFAS biomonitoring in Finland in the light of the current EFSA risk assessment

**Table 1:** Analyzed compounds. The compounds marked with an asterisk (*) are included in the EFSA TWI.

<table>
<thead>
<tr>
<th>Compound</th>
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<tbody>
<tr>
<td>PFHxS</td>
<td>*</td>
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<tr>
<td>PFHpS</td>
<td></td>
</tr>
<tr>
<td>PFOS</td>
<td>*</td>
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<tr>
<td>PFDS</td>
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<tr>
<td>PFHxA</td>
<td></td>
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<td>PFHpA</td>
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<tr>
<td>PFOA</td>
<td>*</td>
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<tr>
<td>PFNA</td>
<td>*</td>
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<tr>
<td>PFDA</td>
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<td>PFUnA</td>
<td></td>
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<tr>
<td>PFDa</td>
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<tr>
<td>PFDoA</td>
<td></td>
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<tr>
<td>PFTrA</td>
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<tr>
<td>PFTeA</td>
<td></td>
</tr>
<tr>
<td>MeFOSAA (not accredited)</td>
<td></td>
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<tr>
<td>EtFOSAA (not accredited)</td>
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</table>

**3. Results:**
The serum levels of PFASs among the 1-year-old children in the DIPP cohort will be available in July 2023 and presented in the Dioxin 2023 conference.

**4. Discussion:**
At the population level, food and especially fish, is the most important source of PFAS intake in Finland. Recently, maximum levels of PFASs in foodstuffs were added into the EU legislation (2022/2388) [4]. Finnish Baltic and freshwater fish are likely to mostly comply to this legislation, confirmed in an ongoing study with results expected in early 2024 [5]. For some individuals or certain areas, dust or drinking water containing PFASs may be a significant source. The EU Drinking Water Directive 2020/2184 [6] sets the maximum level for PFASs (sum of 20 compounds) to 100 ng/l. This maximum level is quite high, and some EU member states have set more strict maximum levels.

The EFSA TWI of 4.4 ng/kg bw per week for PFAS4 is very low compared to intake estimates that have been previously done in Finland for PFASs. It is possible that by consuming modest amounts of fish, the TWI is exceeded (Figure 1). One risk management option to decrease PFAS intake would be to decrease fish consumption, but based on its known net health benefits, this decision should not be made hastily.

Based on PFAS analysis from a small subset of 1-year-old Finnish children (N=54) born in 2005–2006 [7], there is reason to expect that the BMDL10 value of 17.5 ng/ml that EFSA used as the basis for the TWI, will not be exceeded in Finland to the same extent as the TWI (Figure 2). This is likely also because, according to a Swedish study [8], PFAS4 concentrations in breast milk have decreased significantly since 2006. The levels in the breast milk of Finnish mothers will be confirmed in an ongoing study [9].

In a government-led domestic fish consumption promotion program, Finland aims to double the consumption of domestic fish by 2035. This would mean that Finnish people would consume fish according to fish consumption guidelines (at least two times a week, with varying species). The aim of the program is to increase the net benefits that fish consumption has on health. However, if fish consumption in Finland increases as planned, so too will PFAS intake. It is therefore very important to carefully assess the true health effects of PFAS exposure in the Finnish population and to weigh the risk management options. The current study will provide crucial information to do this, with first results available in July 2023.
5. Conclusions:
Previous studies in Finland estimate that the EFSA TWI for $\Sigma$ PFAS4 is likely to be exceeded by most people, while the BMDL10 level in serum of 1-year-old children (17.5 ng/ml), that is critical for health risk of PFAS, is likely not exceeded. Decreasing fish consumption would decrease PFAS intake, but also result in a significant loss of health benefits. With the help of new results in July 2023, the need for risk management measures will be assessed.

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THU-PM1-A2 Maternal Exposure to Per- and Polyfluoroalkyl Substances (PFASs) Associated with the Intrahepatic Cholestasis of Pregnancy (ICP) and Birth Outcomes: a Cross-sectional Case-control Study

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Introduction: Intrahepatic cholestasis of pregnancy (ICP) impairs the release of bile from liver cells, which may result in adverse birth outcomes. While the cause of ICP is not yet clarified, elevated estrogen levels during pregnancy and hepatobiliary transporters have been linked to the condition [1]. Per- and polyfluoroalkyl substances (PFASs) are used in many consumer products and are present in the environment and human serum worldwide. PFASs have been associated with maternal complications and adverse birth outcomes, but their relationship with ICP requires further investigation. Emerging PFAS alternatives, such as 6:2 Cl-PFESA, 4:2 FTS, and ADONA, have similar or slightly lower acute toxicity than older PFASs and have been detected in maternal and umbilical cord serum samples[2]. People living in the Yangtze River Delta, a prosperous region in China with high levels of environmental xenobiotics, have a higher incidence of ICP, making the potential association between ICP and maternal exposure to PFAS a critical environmental health issue. This study aimed to quantify and compare maternal serum concentrations of PFASs, evaluate the association between increased ICP risks, liver function indices, serum hormones, and maternal PFAS exposures, and determine adverse birth outcomes caused by maternal PFAS exposures.

Materials and Methods: The study recruited 78 pregnant women with intrahepatic cholestasis of pregnancy (ICP) and 164 healthy pregnant women as the control group. Maternal blood samples were collected 1-2 days before delivery and analyzed for the presence of various perfluoroalkyl substances (PFASs)[3]. Descriptive characteristics of the participants were collected using questionnaires and from the hospital information system.

Results: Among the targeted PFASs, PFOA and PFOS were the most dominant PFASs, contributing 21% and 23% of the total PFASs, respectively. Comparing the ICP group with the control group, PFOA, PFTrDA, PFHxS, and 8:2 Cl-PFESA showed a significant difference in individual t-test. The concentration of PFOA in the case group was one magnitude higher than that of the control group, while the PFHxS concentration of the case group was one magnitude lower than that of the control group. PFOA and PFOS were positively correlated with all the hepatotoxicity biomarkers and bile acid biomarkers, indicating the possible risk of developing ICP. Four targeted compounds, i.e. PFOA, PFTrDA, PFHxS, and 8:2 Cl-PFESA, showed significant differences between ICP cases and the control population. A multivariable linear regression model was used to investigate the correlations between the hepatotoxicity markers and the associated PFASs, and the results showed that PFOA, PFHxS, 4:2 FTS, ADONA, and 8:2 Cl-PFESA were significantly correlated with the occurrence of ICP.

Discussion and Conclusion: Correlations showed that PFOA may significantly increase the risks of ICP, while PFHxS and emerging PFASs, such as 4:2 FTS and ADONA, appear to have a protective effect on liver and bile function. PFOA, PFDA and 6:2 Cl-PFESA were found to be associated with the upregulation of estrogens, while PFTrDA, PFHxS and 4:2 FTS were negatively correlated with estrogen levels, suggesting that disruption of endocrine homeostasis may contribute to altered ICP risk associated with PFAS exposure. Adverse birth outcomes were found to be correlated with maternal PFOA exposure in the whole study population, but not within the case or control groups. Therefore, from an environmental and public health perspective, maternal PFAS exposure and its association with ICP during pregnancy warrant future attention.

Acknowledgments: FWO junior post-doc fellowships (1270521N), National Natural Science Foundation of China (22276166, 22006010), Zhejiang Shuren University Basic Scientific Research Special Funds (2021ZX017)

Introduction: Perfluoroalkyl substances (PFAS) are a group of environmentally harmful chemicals, which have been and still are in use in many industrial products for their water- and grease-repelling properties, among others. Although the application of the "indicator" substances Perfluorooctane sulfonic acid (PFOS) (Regulation (EU) No. 757/2010) and perfluorooctanoic acid (PFOA) (Regulation (EU) No. 2017/1000) has been banned, many substitutes and precursors are still in use. As these substances are absorbed through plant and animal foods and enter the human body through food contact materials or dust, it is almost impossible to avoid contact with and ingestion of these chemicals. For this reason it is important to minimize exposure to PFAS. Human breast milk was found not to be a major source of PFAS intake for infants. As early as 2006, after the extensive contamination in the Hochsauerland district of Germany became known, the Chemical and Veterinary Analytical Institute Münsterland-Emscher-Lippe (CVUA MEL) tested more than 200 breast milk samples for PFAS. At that time, PFOA and PFOS were found at very low concentrations of 0.1 to 0.2 µg/L in about 150 samples. The transfer of PFAS to breast milk is very low, as shown by Kärrman et al.2 The PFAS ratio between human blood and breast milk is about 100:1. Therefore, unlike the situation for other persistent organic pollutants (POPs), breast milk cannot be considered a suitable bioindicator for PFAS. Since then, however, analytical methods for the determination of PFAS have been further refined. While in 2006 the limits of detection (LOD) and quantification (LOQ) were around 0.05 µg/L, the new, more sensitive methods can reach 1 to 5 ng/L, making it possible to even detect branched PFAS at very low concentrations.

Materials and Methods: Samples of 10 mL homogenized breast milk were weighed into a 50-mL polypropylene (PP) tube. An isotopically labelled internal standard solution was added to the sample (0.5 ng absolute). For extraction 0.1 % ammonia in acetonitrile was added and the samples were shaken at room temperature. The samples were centrifuged and the supernatant was transferred to a 50 mL PP-tube. The extract was evaporated to dryness and dissolved in water. For clean-up, the Freestyle robotic system for PFAS analysis from LCTech with a combined solid-phase extraction cartridge of active carbon and a weak anion exchanger (Strata-X AW, Phenomenex) was used. The target compounds were eluted with 0.1 % ammonia in methanol. The extract was evaporated near to dryness. Afterwards, the residue was redissolved in water and a recovery solution. The purified sample extracts were analyzed using high performance liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). For breast milk an LOQ of 1 to 5 ng/L was achieved for most perfluorocarboxylic acids and perfluorosulfonic acids. The limiting factors of LOQs and LODs were reagent blank values, because PFAS were ubiquitously present in consumables, reagents and in laboratory air.

Results: PFOS, PFOA, perfluorononanoic acid (PFNA) and perfluorohexane sulfonic acid (PFHxS) were detected in 15 breast milk samples. Their concentrations ranged from < LOQ to 0.05 µg/L, with the sulfonic acids being the most abundant. Due to the more sensitive analytical method, the isomeric pattern of the branched isomers is partly recognizable.

Discussion and Conclusion: The developed method allows the simultaneous determination of 22 PFAS in breast milk in a low ng/L range. Furthermore, it is possible to differentiate between the linear substances and the branched isomers of sulfonic acids with the method. The "PFAS 4" (PFOS, PFOA, PFNA and PFHxS) are the predominant PFAS in breast milk. In some samples, the branched isomers in breast milk account for 50% of the total content of the corresponding sulfonic acid. On average, the concentrations are lower -by a factor of 4- than in earlier investigations. In conclusion, the results are in line with recent studies investigating breast milk from the United States3, which concluded from the available PFAS data from 1996 to 2019 that the PFOA and PFOS levels are declining in breast milk. However, previous studies did not differentiate between branched and linear forms, so no data are available. With the more sensitive method, it is possible to differentiate between the isomers and collect data on their uptake and distribution.

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Introduction:
Children are a part of the population that is particularly susceptible to toxicants and, especially, to those that can have a harmful impact on their health due to their young age and their chronical exposure. In this context, we wanted to investigated the exposure of early stage population to commonly known per- and polyfluoroalkyl substances as well as any other new PFAS used as a substitute. For this reason, a non-target methodology was developed for the study of 40 per- and polyfluoroalkyl substances including the banned PFOA and PFOS, among other 18 commonly known PFASs, and the ones considered as new replacement PFASs such as the short TFA, ADONA, GenX, Capstone A and Capstone B, F53B and PFMOBA, among others in urine as a non-invasive matrix.

This study was carried out in collaboration with the INMA-Asturias cohort that includes 173 urine samples from 8-year-old children.

Materials and Methods:
The urine samples were collected by each participant and preserved at -20°C until analysis. Very brief, 100 µl of samples were spiked with a mixture of internal standards and, then, the proteins and part of the salts precipitated with 100 µl of acetonitrile and shacked for 30 min. Afterwards, the samples were centrifuged and the supernatant collected in a LC-vial for further analysis.

The final extracts were analyzed by means of liquid chromatography coupled to high resolution mass spectrometry Orbitrap by full scan at 70000 of FWHM and data dependent scan of the ions at 15000 FWHM. The separation of common PFASs was done with a Hypersil Gold PFP column while the short PFASs were separated in an Atlantis Premier BEH C18 AX column.

Results: The main results showed the presence of common carboxylic acid PFASs such as PFPeA, PFHpA, PFOA, PFNA, PFHxDA, PFUdA and the sulfonates PFHxS, PFOS and PFNS as well as the sulfonamidoacetic acid MeFOSAA at concentrations ranging from 4.9 ng/L (PFNS) to 532 ng/L (PFHxS). In addition, some short chain PFASs have been detected in some samples including TFA. The most frequently detected compound has been the sulfonate PFHxS being present in 39% of samples at quantifiable concentrations followed by the carboxylic acid PFNA in 11% of the samples.

Discussion and Conclusion: The values detected in this work were comparable to those reported for children but at much lower concentration compared with those reported in the literature for adult people [1] and workers from a PFASs manufacture [2]. However, it is important to remark that this study has been focused on 8-years-old children so their exposure to these toxicants has been basically through the diet and, therefore, these concentrations could increase through the years since their exposure could be chronically.

Acknowledgments: This work was supported by "Partnership for the Assessment of Risks from Chemicals – PARC" project co-founded by the European Union (HORIZON-HLTH-2021-ENVHLTH-03: 101057014).

References:
**Introduction:** EPA's Office of Research and Development is rapidly expanding the scientific foundation for understanding and assessing human health risk from PFAS. As part of that research, systematic evidence maps (SEM) have been developed for hundreds of PFAS chemicals, which characterize the toxicological and epidemiological evidence base for over 400 PFAS chemicals representing a range of chemical structures and properties. The PFAS systematic evidence map effort is being expanded to consider the available data for the 'PFAS Universe', of which there are presently 14,735 substances and structures in the EPA Comptox Chemical Dashboard's PFAS Universe list.

**Materials and Methods:** Systematic review methods were used to identify and screen references. A literature search of multiple scientific databases was conducted through December 2022. Intentionally broad Populations, Exposures, Comparators, and Outcomes (PECO) criteria were used to identify references most relevant to human health hazard identification. Additional references that were not PECO-relevant but contained potentially relevant supplemental information were also collected and tagged by supplemental category (e.g., absorption, distribution, metabolism, and excretion (ADME), mechanistic, toxicokinetic, mixtures, exposure characteristics). PFAS with medical anesthetic applications were considered supporting material. Screening was conducted using both manual review and machine-learning software applications. After screening, PECO-relevant references will undergo a focused data extraction to create a literature inventory of study details (e.g., health effect category, exposure route, exposure duration, species) and study quality evaluation (SQE).

**Results:** Over 152,000 references were identified from the literature searches. Of the 14,735 PFAS in the EPA Comptox Chemical Dashboard, literature was only identified for 1,890. Previous SEMs have identified animal and human studies for approximately 80 PFAS. Data extraction and SQE of the identified studies are currently in progress.

**Conclusions:** Thousands of the PFAS assessed in this SEM were data-poor, with most retrieving no literature search results for human health hazard information. This emphasizes the need for robust toxicological and epidemiological information that can inform human health risk assessments of PFAS. This SEM, along with published results from our previous SEMs, provides researchers and regulators a snapshot of the current PFAS human health evidence landscape, as well as a foundation for future systematic reviews. In addition, we hope this evidence map will inform future research and targeted testing to fill data gaps across the diverse PFAS chemical space.

**Acknowledgements:**

The material presented in this abstract has been funded in part by the US EPA under contract 68HERC19D0003 to ICF International.

**References:**

THU-PM1-B1  Children’s Exposure to EH-TBB and Associations with Firemaster® 550 Applications in Residential Furniture

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1 Nicholas School of the Environment, Duke University, Durham, North Carolina 27708, United States

**Introduction:** Flame retardants are chemicals that have been commonly added to furniture, electronics, and construction materials in order to prevent or reduce the spread of fire. 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (EH-TBB) is one component in a flame retardant mixture known as Firemaster® 550 (FM550), which is a replacement for PentaBDE that was banned in 2004 due to health concerns (de Wit. 2002; Stapleton et al. 2012a). In this study, we investigated whether FM550 treatment in furniture contributed to higher levels of exposure in children residing in North Carolina in the United States.

**Material and Methods:** The Toddlers Exposure to semi-volatile organic chemicals (SVOCs) in the Indoor Environment (TESIE) study sought to investigate children’s exposure to SVOCs mixtures in the home. Children in this study were recruited between 2014 to 2016. This analysis included 125 participants from the TESIE cohort who had paired samples of furniture foam, house dust, a silicone wristband, and a composite urine sample. Urine samples were acidified, extracted by solid phase extraction and analyzed using LC-MS/MS to measure tetrabromobenzoic acid (TBBA), the urinary metabolite of EH-TBB (Hoffman et al. 2014). Furniture foam, house dust and handwipes were extracted and analyzed via GC-MS to quantify EH-TBB.

**Results:** Overall, TBBA was detected in 43% of the urine samples, while EH-TBB was detected in 100% of the dust, wristband and handwipe samples. Firemaster® 550 was the most common FR mixture identified in residential foam samples. Statistical analyses revealed that the presence of Firemaster® 550 in furniture was associated with significantly higher average levels of EH-TBB in house dust, wristbands and in hand wipes. Similarly, Firemaster® 550 in furniture were also associated with urinary levels of TBBA, the metabolite of EH-TBB (p<0.01). Higher levels of EH-TBB in the home (e.g., in dust) were correlated with higher levels of EH-TBB on children’s hands as well as higher level of urinary TBBA, indicating higher exposure in children (p<0.001). Analysis of demographic factors revealed that Non-Hispanic Black children had higher urinary TBBA levels compared to Hispanic and Non-Hispanic White Children (p<0.05).

**Discussion: and Conclusion:** Due to the limited data available on exposure to EH-TBB, more studies are needed to measure urinary metabolite levels in both adults and children. As this was a small study, larger studies are needed to determine if exposure levels are consistent in the broader population and in different regions. Including a larger number of participants with different age ranges and more diversity race would be valuable to understanding variables contributing to exposure. Additionally, studies are needed to investigate how stable TBBA is in the body and how variable levels are in urine from day to day.

**Acknowledgments:** Funding for this research was provided by grants from the US Environmental Protection Agency (Grant 83564201), the NIEHS (R01 ES016099) and Housing and Urban Development (NCHHU0062). We also thank our participants for opening their homes to our study team and helping us gain a better understanding of children’s exposures to SVOCs.

**References:**
Introduction: Plastics often consist of a complex mixture of unreacted intermediates, monomers, and additives such as dyes, fillers, antioxidants, flame retardants (FRs), UV stabilizers, surfactants, and plasticizers, all to improve or modify the performance of the product. Many of these additives migrate during use and/or pose threats to human and environmental health during production and disposal. Although the EU has banned organohalogen FRs in electronics displays, few policies in the US regulate the use of FRs. Brominated compounds used to flame retard electronics have been found in consumer products purchased in Africa, Europe, and Asia due to lack of regulation and poor recycling practices. To investigate if U.S. consumer products that do not require flame retardancy are contaminated with regulated/unregulated FRs, a selection of food contact items, hair accessories, kitchen utensils, and toys was analyzed for the presence of 21 FRs.

Materials and methods: 200 black plastic products (food serviceware, n=25; hair accessories, n=30; kitchen utensils, n=109; toys, n=36) were purchased from online retailers and local stores in and around Seattle, USA, from 2020 to 2022. In total 20 plastic consumer products (food serviceware, n=2; hair accessories, n=1; kitchen utensils, n=9; toys, n=8) with at least 50 parts per million of Br as determined by XRF were selected. Approximately 50 mg of cryomilled plastic samples were extracted and desolved using toluene/DCM mixture. Prior analyses labeled standards (13C12-HBCDD, 13C12-TBBPA, 13C12-BDE209, 13C12-BDBPE, 13C12-BTBPE, 13C18-TTBP-TAZ, 13C6-246-TBP and TPHP-d15) were added. Twenty-one BFRs and PFRs were analyzed using a high-resolution quadrupole time-of-flight (qTOF) mass spectrometer (MS) (Compact, Bruker, Bremen, Germany) connected to a LC 1260 HPLC (Agilent, Amstelveen, the Netherlands). The mobile phase consisted of HPLC water and methanol using gradient conditions. Measurement of PFRs was performed in positive atmospheric pressure chemical ionization (APCI) mode and the BFRs in negative APCI mode.

Table 1. FRs and plasticizers detected in the consumer products. Including detection frequency and concentration ranges.

<table>
<thead>
<tr>
<th>Compound</th>
<th>CAS No.</th>
<th>Detection frequency</th>
<th>Range</th>
<th>Product with highest level</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBBPA</td>
<td>79-34-7</td>
<td>75%</td>
<td>5 - 1370</td>
<td>Sushi container</td>
</tr>
<tr>
<td>BOE 209</td>
<td>1163-19-5</td>
<td>70%</td>
<td>50 - 11900</td>
<td>Sushi container</td>
</tr>
<tr>
<td>2,4,6-TBP</td>
<td>118-79-7</td>
<td>70%</td>
<td>0.8 - 310</td>
<td>Peeler</td>
</tr>
<tr>
<td>DBDPE</td>
<td>8483-53-9</td>
<td>60%</td>
<td>19 - 2000</td>
<td>Pirate con nedation</td>
</tr>
<tr>
<td>BDP</td>
<td>5945-32-5</td>
<td>60%</td>
<td>5 - 420</td>
<td>Pirate con nedation</td>
</tr>
<tr>
<td>RDP</td>
<td>57583-94-7</td>
<td>60%</td>
<td>9.3 - 15000</td>
<td>Party beads</td>
</tr>
</tbody>
</table>

Results: The Br content in the plastic consumer products measured with XRF ranged from 51 to 18600 ppm. BFRs and PFRs were detected in 17 of the 20 consumer products, and the BFR levels correlated well with the Br content in all but 3 cases. TBBPA was the most common FR, detected in 75% of the consumer products followed by BDE-209 (70%), 2,4,6-TBP (70%), DBDPE (60%), BDP (60%), RDP (60%), TPHP (55%) and TTBP-TAZ (50%) (Table 1). The highest BFR levels were found for BDE-209 with concentrations up to 11900 mg/kg detected in a sushi container (Table 1). In 70% of the plastic products the levels exceed the unintentional trace contaminant limit of 10 mg/kg for decaBDE (BDE-209) as introduced by the EU in July 2021 for mixtures and articles (European Commission, 2019). In 60% of plastic consumer products, more than 6 different FRs were detected. The decaBDE replacements, DBDPE and TTBP-TAZ, were also frequently detected, indicating that these compounds have also made their way into the waste electrical and electronic equipment (WEEE) stream and subsequently into other, non-electronic household products.

Discussion and Conclusion: The finding of multiple hazardous FRs in plastic consumer products is worrying and points to the need for elimination of hazardous additives to support the move to a circular economy. Besides various BFRs previously detected in electronics displays, the finding of other FRs can be seen as a confirmation of WEEE-specific contamination as none of the consumer products analyzed needed to contain FRs. The appearance of WEEE fractions in plastic consumer products is a result of poor recycling practices and lack of FR regulation. The finding of elevated FR levels in the toys is of especially high concern as these can easily migrate into the saliva during mouthing by young children. Ongoing research is further investigating the presence of other plastic additives in these consumer products using the non-target screening approach.

Acknowledgments: Special recognition to Nancy Uding who initiated this research.

THU-PM1-B3  Dust-bound replacement flame retardants in indoor environments: homes, schools, and café/bar/restaurants

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Introduction: Flame retardant chemicals are commercially produced in high volumes since the 1970s due to the regulatory flame retardancy requirements of electrical and electrical devices, textiles, construction materials, polyurethane foams, and floorings. The demand for non-PBDE flame retardants was increased after the regulatory restrictions on PBDEs. Increasing production volumes of replacement flame retardants such as organophosphate esters (OPEs), non-BDE brominated flame retardants, and commercial dechlorane plus mixtures (AFRs) resulted in the abundantly occurring replacement FRs in indoor environments. Settled dust-bound OPEs and AFRs were determined in indoor and outdoor environments of homes, schools, and C/B/Rs in this study.

Materials and Methods: Indoor settled dust samples were collected from homes (n=21), schools (n=21), and café/bar/restaurants (n=13) using a vacuum cleaner from September 2019 to February 2020 in İzmir-Turkey. Outdoor settled dust samples were simultaneously collected from non-soil surfaces. Collected samples were sieved (mesh size: 500 µm) and ultrasonically extracted using acetone:hexane mixture (v:v, 1:1) after overnight soaking. Extracts were concentrated using a rotary evaporator and cleaned with silica-bedded solid phase extraction cartridges, and solvent was exchanged to isooctane. While OPEs were analyzed in GC-EI-MS, alternative flame retardants were analyzed in GC-NCI-MS. The targeted compounds were 11 OPEs and 11 AFRs.

Results: Settled dust-bound AFR concentrations in indoor environments of homes, schools, and C/B/Rs were determined to be 886, 1328, and 1014 ng/g, respectively. Indoor dust AFR concentrations were dominated by α- and β isomers of 1,2-Dibromo-4-(1,2-dibromoethyl)cyclohexane (ΣDBE-DBCH) in homes (ΣDBE-DBCH,Homes=678 ng/g) and C/B/Rs (ΣDBE-DBCH,C/B/R=813 ng/g) while 1,2-bis(2,4,6-tribromophenoxy)ethane (CBTBPE=585 ng/g) and hexabromocyclododecane (CHBCDD=449 ng/g) dominated in schools. The average indoor settled dust ΣOPE concentrations in homes, schools, and C/B/Rs were determined to be 10516, 22275, and 14943 ng/g, respectively. While tributoxyethyl phosphate (TBOEP) levels significantly dominated the indoor settled dust-bound ΣOPE concentrations in homes with an average of 6084 ng/g, that averages in schools and C/B/Rs were 8040 and 3860 ng/g, respectively. Alkyl OPE concentrations in homes, schools, and C/B/Rs were determined to be higher than those of chlorinated and alkyl OPEs with concentration fraction averages of 51, 44, and 36%, respectively. The averages for chlorinated OPEs were 25, 21, and 34%, and for aryl OPEs were 24, 35, and 30%, respectively. Indoor/outdoor concentration ratios of all targeted flame retardants were determined to be higher than 1.

Discussion and Conclusion: Settled dust-bound replacement flame retardant concentrations in schools were determined to be higher than those in homes and C/B/Rs. OPE concentrations were significantly higher than AFR concentrations at all sampling points which might be due to the difference in their source strengths in indoor environments. The most abundant replacement flame retardants in indoor environments of houses, schools, and C/B/Rs were determined to be DBE-DBCH, BTBPE, and HBCDD among AFRs and TBOEP in OPEs. Relatively high indoor dust concentrations of OPEs indicated that high exposures might occur for students considering their lower body weights and higher accidental ingestion rates compared to adults.

Acknowledgments: This study was supported by The Scientific and Technological Research Council of Turkey (TUBITAK) Grant #118Y142.

References:
Introduction: The International Space Station (ISS) is a unique indoor environment inhabited by humans for over twenty years. Firstly, it contains many putative sources of persistent organic contaminants (POCs) – e.g. electrical and electronic equipment, insulation foam, and other commercially available plastic products – within a relatively small space. Secondly, air inside the ISS is constantly recirculated with HEPA filtration. Additionally, high levels of ionizing radiation can cause accelerated ageing of materials, including breakdown of plastic goods into micro and nanoplastics that can become airborne in the microgravity environment. Furthermore, the original fluorescent lighting was only recently exchanged with LEDs, so photolytically-mediated degradation of some compounds may occur. We hypothesized that the concentrations and relative abundance of PBDEs, HBCDD, NBFRs, OPEs, PFAS, and PCBs in ISS dust differ notably from those in dust from terrestrial indoor microenvironments.

Materials and Methods: To test our hypothesis, we measured the concentrations of a range of POCs in dust collected from the ISS in 2019 within the Divert Unwanted Space Trash (DUST) experiment. The dust was collected in vacuum cleaner bags during the weekly cleaning of the ISS HEPA filters. On receipt of an aliquot of this dust at Birmingham, the sample was passed through a 125 µm sieve prior to analysis via solvent extraction, SPE, and either GC-MS for PBDEs, OPEs, and NBFRs, or LC-QTOF-MS for PFAS and HBCDDs.

Results: Table 1 provides concentrations of selected target compounds in the ISS dust sample, with data from studies of US house dust to provide context.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Concentration in ISS dust (ng/g)</th>
<th>Median (range) concentration in US house dust (ng/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDE-47</td>
<td>8,400</td>
<td>420 (not stated (ns)-130,000)</td>
</tr>
<tr>
<td>BDE-99</td>
<td>27,000</td>
<td>580 (ns-140,000)</td>
</tr>
<tr>
<td>BDE-183</td>
<td>230</td>
<td>15 (&lt;2-1,100)</td>
</tr>
<tr>
<td>BDE-209</td>
<td>18,000</td>
<td>910 (ns-990,000)</td>
</tr>
<tr>
<td>HBBz</td>
<td>530</td>
<td>4.7 (&lt;1-1,100)</td>
</tr>
<tr>
<td>EH-TBB</td>
<td>790</td>
<td>1,200 (&lt;5-130,000)</td>
</tr>
<tr>
<td>α-HBCDD</td>
<td>2,300</td>
<td>240 (ns-17,000)</td>
</tr>
<tr>
<td>β-HBCDD</td>
<td>1,000</td>
<td>38 (ns-14,000)</td>
</tr>
<tr>
<td>γ-HBCDD</td>
<td>95,000</td>
<td>89 (ns-300,000)</td>
</tr>
<tr>
<td>TPhP</td>
<td>15,700</td>
<td>8,100 (ns-110,000)</td>
</tr>
<tr>
<td>PCB-52</td>
<td>19</td>
<td>6.2 (1.7-28)</td>
</tr>
<tr>
<td>PCB-101</td>
<td>190</td>
<td>8.7 (1.9-29)</td>
</tr>
<tr>
<td>PFOS</td>
<td>23</td>
<td>200 (&lt;8.9-12,000)</td>
</tr>
<tr>
<td>PFOA</td>
<td>2,600</td>
<td>140 (&lt;10-1,960)</td>
</tr>
</tbody>
</table>

Discussion and Conclusion: While concentrations of all target compounds frequently exceed the median in US house dust; ISS dust concentrations were generally within the terrestrial range. γ-HBCDD is the dominant diastereomer (96.6% SHBCDD). This matches with the commercial mixture and contrasts with the diastereomer distribution observed in most house dust samples (where γ-HBCDD is typically ~60-70% SHBCDD). This suggests conditions inside the ISS do not favour photolytically-mediated formation in dust of α-HBCDD. Also of note, the concentration of perfluorooctanoic acid (PFOA) in ISS dust (2,600 ng/g) exceeds the maximum reported (1,960 ng/g) in a 2008 survey of dust from US child daycare centers and homes. Our findings can inform future material choices for manned spacecraft such as the ISS.
Introduction: The North American Great Lakes basin has a myriad of land uses. A long history of manufacturing in the region has led many synthetic organic pollutants to become ubiquitous in the environment, including PBDEs (polybrominated diphenyl ethers), PCBs (polychlorinated biphenyls), legacy pesticides, and PFAS. Areas with the highest concentrations of these pollutants are dubbed "Areas of Concern," or AOCs by the United States Environmental Protection Agency. An AOC is a geographic region "where significant impairment of beneficial uses has occurred as a result of human activities at the local level."

One such AOC is the River Raisin, for which remediation was completed in 2016. However, there are still fish consumption advisories as well as bird and wildlife reproduction problems. Our study examines concentrations of legacy pollutants in 199 herring gull blood serum samples from a site at the mouth of the River Raisin, two other known areas of contamination, and two reference sites.

Materials and Methods: Herring gull blood serum samples were collected between 2010 and 2021. An aliquot of the herring gull serum sample was liquid-liquid extracted using hydrochloric acid, isopropanol, and 1:1 hexane:MTBE (v/v). The combined supernatants were neutralized, concentrated to 1 mL, and cleaned on a solid-phase chromatography column. Three fractions of different polarity were collected. The first two fractions were injected on the GC-MS for flame retardant analysis, and on the GC-ECD for legacy pesticide and PCB analysis.

Results: PBDEs were detected in all herring gull serum samples, with median concentrations ranging from 4.5 to 8.2 ng/g ww. Based on the results of an ANOVA, the ΣPBDE concentrations at DE and SBCDF are significantly higher than the concentrations at BELL and SBLCI. Syn- and anti-DP (Dechlorane Plus) were also analyzed, and fanti ranges were within reported ranges of the commercial mixture. Novel flame retardants were infrequently detected.

Median concentrations of total legacy pesticides (ΣPest) across all five locations are between 9.7 and 29.2 ng/g ww. There were no significant temporal trends in the concentration for either ΣPest or individual pesticide. The median concentrations of five DDT isomers and metabolites (ΣDDTs) across all five sites range from 6.4 to 18.3 ng/g. Over all five sites, the concentrations of ΣDDTs are significantly decreasing (p = 0.005) with a halving time of 8.2 ± 4.3 years.

PCBs were also detected in all herring gull samples, with median concentrations ranging from 27.8 to 161.2 ng/g ww. Notably, 40-77% of the concentration of contaminants in the herring gulls comes from PCBs. Samples from the site at the mouth of the River Raisin had the highest concentration of PCBs.

PFAS were detected in all herring gull serum samples with PFOS dominating.

Discussion and Conclusion: Concentrations of Penta-BDE, DDTs, and PCBs are significantly decreasing at 10.6 ± 4.9 years, 8.2 ± 4.3 years, and 5.9 ± 3.1 years respectively, which is generally in agreement with atmospheric data over the same region. Additionally, the lack of detection and trend of many pesticides agrees with their slow elimination over the Great Lakes Region. The high concentration of PCBs at the River Raisin site suggests that even though remediation has finished, continuous monitoring is necessary for highly impacted wildlife in the area.

References:
1. Introduction:
Huge amounts of general industrial solid wastes (IW) in China from industrial production have attracted widespread attention from researchers and the public. Statistical data shows that the amount of IW generated in China ramped up to 3.67 billion tons in 2020, with an average growth rate of 6.6% (from 2010 to 2020). Accordingly, the Chinese government has promulgated policies to promote the resourceful use of IW, and further reduce the stock of bulk IW in an orderly manner.

IW comprises inorganic/organic heterogeneous materials, whose properties are closely related to their source plants. Without proper disposal methods, IW (e.g., from the textile and food industry) would generate excessive polychlorinated dibenzo-\textit{p}-dioxins and dibenzofurans (PCDD/Fs) emission during the thermochemical process. Co-incinerating municipal solid waste (MSW) with IW in thermal treatment plants can be a solution. The storage pool, disposal capacity, heat recovery facility, and air pollution control system (APCS) of MSWI plants can be fully utilized to synergize IW by co-disposal. Leading waste disposal enterprises in China (e.g., EB Environment, SUS Environment) have launched nearly 20 co-disposal projects during 2021-2022. Prior studies suggested that organic IW might lead to higher PCDD/Fs emission due to higher chlorine (Cl) content. Different types of fuels might result in distinct formation pathways of PCDD/Fs, whose generation could be tailored and suppressed, ensuring emission compliance. However, the emission and formation characteristics of PCDD/Fs from MSW co-incinerating IW in full-scale facilities are relatively underexplored.

In this study, the emission of toxic PCDD/Fs in flue gas and fly ash from a full-scale MSW incinerator is determined under co-disposal conditions (80 wt% MSW and 20 wt% IW (textile industry or food industry). Under the operation of APCS, the removal efficiency of PCDD/Fs is correspondingly identified in different scenarios. The emission characteristics of 136 kinds of tetra-through octa-chlorinated dibenzo-\textit{p}-dioxin and dibenzofurans (136 PCDD/Fs) are analyzed and compared. Accordingly, the potential influence of MSW co-incinerating IW on the formation pathway of PCDD/Fs is investigated.

2. Materials and method:
2.1 Studied plant
The research is conducted at a full-scale MSWI plant. The incinerator system is composed of a waste pool, grate incinerator, selective non-catalytic reduction (SNCR), semi-dry spray neutralizer, activated carbon, fabric filter and chimney. In detail, MSW with IW is first transported into the waste pool and then composted for 3-5 days. Afterward, MSW with 20 wt% IW is pre-treated by magnetic separation for latent metal recovery. The pre-separated fuel is mixed and grabbed into the 400 t/day grate incinerator, and is combusted thoroughly with an average furnace outlet temperature of 1050 °C.

2.2 Sample
Flue gas and fly ash samples of PCDD/Fs are parallely extracted for MSWI and IW co-incineration conditions. The procedures are illustrated below. Before sampling, the PCDD/F surrogate standards are added to the XAD-2 resin to check the sampling efficiency. Following HJ77.2-2008 and HJ77.3-2008, the effluent gas samples of PCDD/Fs are parallely extracted by the gas sampler (ZR-3720, Junray, China) and gas analyzer (DX-4000, Gasmet, Finland) in the inlet and outlet of APCS, for 1 hour at each sampling time. Meanwhile, the online continuous emission monitoring system (CEMS, SDL Technology, China) and online smoke monitor (3012HC, Laoying, China) are adopted for major air pollutant measurement.

2.3 Analysis
Briefly, pre-treatment includes spiking with internal standards, Soxhlet extraction, clean-up with silica gel column and basic-alumina column, and spiking with recovery standards. Afterward, purified samples are analyzed by GC/MS (JMS-800D, JEOL, Japan). The recovery rates of the PCDD/Fs range from 43.2% to 109.7%, meeting the requirement (30% to 130%) of EPA Method 1613. Following this, NATO/CCMS factors are used to calculate international toxic equivalents. All calculated concentrations of PCDD/Fs and major air pollutants are also normalized to 11% O2, 273.15K, and 100 kPa (GB 18485-2014).

3. Results and Discussion:
3.1 Co-disposal influence on toxic PCDD/Fs
The total and I-TEQ concentrations of toxic PCDD/Fs in flue gas from MSWI at the boiler outlet (BO) are 1.86 ng/Nm3 and 0.14 ng I-TEQ/Nm3, which are surprisingly higher than 0.89 ng/Nm3 and 0.07 ng I-TEQ/Nm3 of PCDD/Fs in primary flue gas from IW#1 co-disposal, as well as 1.15 ng/Nm3 and 0.12 ng I-TEQ/Nm3 of PCDD/Fs from IW#2 co-disposal. Accordingly, I-TEQ
concentrations of toxic PCDD/Fs are 0.005 ng I-TEQ/Nm\(^3\) (MSW) and 0.002/0.003 ng I-TEQ/Nm\(^3\) (IW\#1/#2), implying a similar decreasing tendency of gas-phase PCDD/Fs from flue gas through the APCS. The removal efficiencies of toxic PCDD/Fs by APCS are 96.3\% (MSW) and 97.3\%/97.0\% (IW\#1/#2 co-disposal). In contrast, mean contents of toxic PCDD/Fs in fly ash (FA) have a reversed trend, reaching 490.36 ng/kg (37.47 ng I-TEQ/kg) of IW\#1 co-disposal and 327.67 ng/kg (21.75 ng I-TEQ/kg) of IW\#2, which is significantly higher than 284.20 ng/kg (17.00 ng I-TEQ/kg) of MSWI. Therefore, the implementation of IW co-disposal might strengthen the pollutant enrichment of fly ash in fume and slightly raise the temperature (200-500\) of residue gas over reheater and economizer, which enhances the low-temperature heterogeneous synthesis of PCDD/Fs.

The distribution of toxic PCDD/F congeners on the total/I-TEQ concentration level is displayed in Figure 1. For toxic PCDD congeners, co-incinerating IW fails to significantly affect the composition of isomers, as 1,2,3,4,6,7,8-HpCDD and OCDD are the major contributors to total concentrations. For toxic PCDF congeners, co-incinerating IW slightly influences the isomer distribution, as 1,2,3,4,6,7,8-HpCDF and OCDF in IW\#1/#2 co-disposal condition decreases to 19.3\%/17.3\% and 15.0\%/17.2\% (BO), as well as 21.2\%/20.6\% and 9.5\%/13.8\% (FA), respectively. For the overall toxic PCDD/Fs, 1,2,3,4,6,7,8-HpCDD, OCDD, 1,2,3,4,6,7,8-HpCDF, and OCDF are still the major (>10\%) in primary flue gas and fly ash for MSWI normal operation condition and IW co-disposal conditions. However, fractions of high-chlorinated isomers such as 1,2,3,4,6,7,8-HpCDD and OCDD ramp up in gas from CH, possibly caused by selective adsorption of sprayed activated carbon in fabric filter [56]. Additionally, due to different international toxic equivalency factors (I-TEF) of toxic PCDD/F isomers, 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 2,3,7,8-TCDF, and 2,3,4,7,8-PeCDF contribute most to the sum I-TEQ concentration of toxic PCDD/Fs. In summary, co-incinerating IWS (textile and food) would not significantly change the distribution pattern of toxic PCDD/F isomers.

![Figure 1. Total (a, c, e) and I-TEQ (b, d, f) fractions (%) of toxic PCDD/F congeners in MSWI and IW co-disposal conditions](image-url)
3.2 Formation pathways of PCDD/Fs

As reaction positions are essential for PCDD/Fs formation, Table 1 summarizes the total concentrations of individual homologues (e.g., TCDD, PeCDD, et al.) of 136 PCDD/F congeners in different phases. In the process of co-incinerating IW#1 and #2, the sum concentration of 136 PCDF/F isomers from flue gas in BO changes unexpectedly from 12.71 ± 4.47 ng/Nm³ (MSW) to 6.83 ± 2.48 ng/Nm³ and 8.71 ± 2.13 ng/Nm³ (co-incinerating IW#1 and #2), respectively. The results indicate that blending IW of higher Cl content would not strengthen PCDD/F outputs in the primary flue gas. However, PCDD/F contents in FA increase substantially from 895.92 ± 455.17 ng/kg (MSW) to 2183.25 ± 962.09 ng/kg and 1200.42 ± 375.40 ng/kg (co-incinerating IW#1 and #2). The trend is in alignment with toxic PCDD/Fs, possibly due to the higher source strength of Cl in fuel and the enrichment of PCDD/Fs to the surface of the granular phase.

Residues of chlorine-containing compounds and hydrocarbons are catalytically oxidized and chlorinated into PCDD/Fs (de novo synthesis), in which PCDFs are generated far more than PCDDs. To identify the formation pathway of PCDD/Fs for different co-disposal conditions, the ratio of integral PCDDs to PCDFs in BO/FA (Table 1) is calculated, which turns from 0.50/0.85 (MSW) to 0.79/0.35 (co-incinerating IW#1) and 0.52/0.50 (co-incinerating IW#2). The significantly higher portions of PCDFs than PCDDs indicate that de novo synthesis rather than precursor synthesis is the domination of PCDD/Fs formation. Concretely, precursor synthesis forms the majority of PCDFs [29], [59]. To further verify the statement, the integral ratio of PCDDs to PCDFs is calculated likewise based on the PCDDs and PCDFs concentrations/synthesis forms the majority of PCDFs [29], [59]. To further verify the statement, the integral ratio of PCDDs to PCDFs is calculated likewise based on the PCDDs and PCDFs concentrations/synthesis forms the majority of PCDFs [29], [59]. To further verify the statement, the integral ratio of PCDDs to PCDFs is calculated likewise based on the PCDDs and PCDFs concentrations/synthesis forms the majority of PCDFs [29], [59]. To further verify the statement, the integral ratio of PCDDs to PCDFs is calculated likewise based on the PCDDs and PCDFs concentrations/synthesis forms the majority of PCDFs [29], [59]. To further verify the statement, the integral ratio of PCDDs to PCDFs is calculated likewise based on the PCDDs and PCDFs concentrations/synthesis forms the majority of PCDFs [29], [59]. To further verify the statement, the integral ratio of PCDDs to PCDFs is calculated likewise based on the PCDDs and PCDFs concentrations/synthesis forms the majority of PCDFs [29], [59]. To further verify the statement, the integral ratio of PCDDs to PCDFs is calculated likewise based on the PCDDs and PCDFs concentrations/synthesis forms the majority of PCDFs [29], [59]. To further verify the statement, the integral ratio of PCDDs to PCDFs is calculated likewise based on the PCDDs and PCDFs concentrations/synthesis forms the majority of PCDFs [29], [59].

<table>
<thead>
<tr>
<th>PCDD/F</th>
<th>MSW BO (ng/Nm³)</th>
<th>IW#1 BO (ng/Nm³)</th>
<th>IW#2 BO (ng/Nm³)</th>
<th>MSW CH (ng/Nm³)</th>
<th>IW#1 CH (ng/Nm³)</th>
<th>IW#2 CH (ng/Nm³)</th>
<th>MSW FA (ng/kg)</th>
<th>IW#1 FA (ng/kg)</th>
<th>IW#2 FA (ng/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCDD</td>
<td>1.89 ± 0.65</td>
<td>1.37 ± 0.61</td>
<td>1.59 ± 0.44</td>
<td>0.062 ± 0.053</td>
<td>0.032 ± 0.014</td>
<td>0.039 ± 0.012</td>
<td>70.71 ± 39.55</td>
<td>103.94 ± 39.15</td>
<td>18.68 ± 77.36</td>
</tr>
<tr>
<td>PeCDD</td>
<td>0.96 ± 0.25</td>
<td>0.62 ± 0.10</td>
<td>0.64 ± 0.08</td>
<td>0.061 ± 0.059</td>
<td>0.020 ± 0.016</td>
<td>0.028 ± 0.010</td>
<td>69.61 ± 18.85</td>
<td>108.52 ± 56.29</td>
<td>54.42 ± 47.26</td>
</tr>
<tr>
<td>HxCDD</td>
<td>0.69 ± 0.19</td>
<td>0.56 ± 0.34</td>
<td>0.39 ± 0.03</td>
<td>0.044 ± 0.036</td>
<td>0.017 ± 0.007</td>
<td>0.026 ± 0.015</td>
<td>78.56 ± 76.74</td>
<td>92.74 ± 88.91</td>
<td>76.79 ± 47.26</td>
</tr>
<tr>
<td>HpCDD</td>
<td>0.38 ± 0.10</td>
<td>0.24 ± 0.03</td>
<td>0.18 ± 0.03</td>
<td>0.027 ± 0.014</td>
<td>0.013 ± 0.007</td>
<td>0.024 ± 0.008</td>
<td>96.05 ± 77.36</td>
<td>122.72 ± 96.54</td>
<td>73.53 ± 17.66</td>
</tr>
<tr>
<td>OCDD</td>
<td>0.31 ± 0.10</td>
<td>0.21 ± 0.03</td>
<td>0.18 ± 0.07</td>
<td>0.025 ± 0.011</td>
<td>0.014 ± 0.007</td>
<td>0.024 ± 0.008</td>
<td>96.32 ± 67.37</td>
<td>133.14 ± 57.38</td>
<td>114.39 ± 31.46</td>
</tr>
<tr>
<td>TCDF</td>
<td>3.63 ± 0.74</td>
<td>2.50 ± 0.56</td>
<td>3.65 ± 1.11</td>
<td>0.045 ± 0.032</td>
<td>0.026 ± 0.013</td>
<td>0.038 ± 0.008</td>
<td>224.17 ± 119.79</td>
<td>946.75 ± 172.15</td>
<td>435.82 ± 367.96</td>
</tr>
<tr>
<td>PeCDF</td>
<td>1.90 ± 1.38</td>
<td>0.69 ± 0.45</td>
<td>1.15 ± 0.41</td>
<td>0.036 ± 0.028</td>
<td>0.010 ± 0.009</td>
<td>0.016 ± 0.002</td>
<td>117.96 ± 50.62</td>
<td>347.85 ± 132.10</td>
<td>185.01 ± 49.13</td>
</tr>
<tr>
<td>HxCDF</td>
<td>1.53 ± 1.21</td>
<td>0.37 ± 0.33</td>
<td>0.56 ± 0.20</td>
<td>0.033 ± 0.025</td>
<td>0.011 ± 0.001</td>
<td>0.016 ± 0.002</td>
<td>85.55 ± 60.13</td>
<td>214.22 ± 111.02</td>
<td>111.62 ± 76.30</td>
</tr>
</tbody>
</table>

Table 1. Concentrations and partitions of 136 PCDD/F homologues in MSWI and IW co-disposal conditions
THU-PM1-C1  Emission, Partition, and Formation Pathway of PCDD/Fs during Co-disposal of Industrial Waste with Municipal Solid Waste

<table>
<thead>
<tr>
<th></th>
<th>BO (ng/Nm³)</th>
<th>CH (ng/Nm³)</th>
<th>FA (ng/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MSW</td>
<td>IW#1</td>
<td>IW#2</td>
</tr>
<tr>
<td>HpCDF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>±0.84</td>
<td>±0.03</td>
<td>±0.01</td>
</tr>
<tr>
<td>OCDF</td>
<td>0.40</td>
<td>0.07</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>±0.27</td>
<td>±0.03</td>
<td>±0.11</td>
</tr>
<tr>
<td>Sum</td>
<td>12.71</td>
<td>6.83</td>
<td>8.71</td>
</tr>
<tr>
<td></td>
<td>±4.47</td>
<td>±2.48</td>
<td>±2.13</td>
</tr>
<tr>
<td>PCDD/Fs</td>
<td>0.50</td>
<td>0.79</td>
<td>0.52</td>
</tr>
<tr>
<td>dCDP/PCDD/Fs</td>
<td>5.13</td>
<td>4.82</td>
<td>4.71</td>
</tr>
</tbody>
</table>

Figure 2. PCDD/Fs congener profiles in MSWI and IW co-disposal conditions
THU-PM1-C1  Emission, Partition, and Formation Pathway of PCDD/Fs during Co-disposal of Industrial Waste with Municipal Solid Waste

4. Conclusions:
The MSW is co-incinerated with 20 wt% IW (IW#1 of the textile mill or IW#2 of the food industry, respectively) in a full-scale MSW incinerator to explore the emission characteristics and formation pathways of PCDD/Fs. The estimation of PCDD/F concentrations and analysis of PCDD/Fs isomer signature are comprehensively conducted and studied. Statistical methods dissect adequate experimental data sets, and the results show that:

MSW co-incinerating IW lowers the emission of toxic PCDD/Fs in flue gas from both BO and CH but elevates toxic PCDD/F contents in FA, all of which satisfy the national limitation standard (GB18485-2014 and HJ 1134-2020).

IW co-disposal influences less on the distribution patterns of 136 PCDD/F isomers yet causes the PCDD/Fs migration to FA, possibly attributed to Cl increment in IW.

De novo synthesis is always dominant in all conditions, which IW co-disposal strengthens. Co-incinerating IW#1/#2 slightly decreases the fractions of CP-route, and DF chlorination yet enhances DD chlorination.

5. Acknowledgment:
This study is supported by the National Key Research and Development Program of China (2020YFC1910100).

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Formation, Sources and Control
S. Lomnicki & J. Petrlik

THU-PM1-C3 Persistent Organic Pollutants and Heavy Metals in Surrounding of Disposal Sites of Waste Incineration Ash in Southern Taiwan

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1. Introduction:
Waste incinerators are listed among major sources of unintentionally produced persistent organic pollutants (POPs) in Annex C to the Stockholm Convention. Large amounts of polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) are released into the environment through waste incineration (WI) residues like fly ashes and other air pollution control (APC) residues and/or bottom ash. These residues were studied extensively in Taiwan due to their significant contribution to toxic chemical releases. This report examines how POPs from WI residues can contaminate the environment, using existing literature and sampling from four sites in southern Taiwan.

Solid WI produces residues in different forms, estimated to be between 25% and 35% (sometimes up to 40%) of the original weight of waste input. Bottom ash makes up the majority, ranging from 20-30% of the original waste on a wet basis. APC residues account for 2-5% of waste input mass on a wet basis (1-3% for fly ash) but contain higher concentrations of toxic chemicals such as PCDD/Fs, however a lower concentration of polybrominated dibenzo-p-dioxins and dibenzofurans (PBDD/Fs) and PFS compared to bottom ash. The global production of fly ash from burnt waste is estimated to be 6.85 million tonnes annually, assuming 3% is created. However, the actual amount may be lower due to underutilization of the capacity of municipal solid waste incinerators (MSWIs). It is estimated that globally, 14-15 kg TEQ of PCDD/Fs are found in WI ash each year. There were 26 MSWIs, and/or W-t-E plants in Taiwan, with a designed capacity of 24,650 tons/day in 2008. Among these, 24 are operational, while two are not due to protests. Our study focuses on waste incinerators mainly located in the south of Taiwan and one near Taipei.

Taiwanese landfills are often built as walled blocks, sometimes using fly ash monoliths from incineration plants. Fly ash from the Kanding Plant is buried in Fanglya landfill, while Yan Chao and Kangshan landfills received fly ash from incineration plants in Kaohsiung. Bottom ash from incinerators in Pingtung, Kaohsiung, Tainan, and Taichung was illegally dumped in Ancing road. The Yan Chao landfill is built from stabilized fly ash monoliths. The Kangshan landfill is fenced with concrete walls and was closed in 2017, with only 5.5 ha used to store fly ash monoliths. The Fang Lyao landfill is a municipal waste landfill, where WI fly ash in big bags and bottom ash were stored in a dedicated part of the facility. Ancing Road - WI (bottom) ashes were dumped in the middle of the ponds used in part by fishermen. More details can be found in a broader report published this year.

2. Materials and Methods:
We sampled three secured landfills - Yan Chao (6 samples), Kangshan (4 samples), and Fang Lyao (3 samples) where municipal WI ash was or is stored, as well as a site in Annan where bottom ash was dumped into ponds (Ancing Road, 3 samples). Soil, sediment, and dust samples were collected at all sites, and fly ash or bottom ash samples were collected where possible. Additional dust samples included limited quantities of shrimps from the pond near Ancing Road and free-range chicken eggs from a farm 0.5 km away from the landfill in Fang Lyao. Sampling was conducted by researchers from Tainan Community University, Taiwan Watch Institute, and Arnika in January 2017. We also used a fly ash sample (1 sample) from Mucha Waste Incinerator for comparison (Taipei). Six of all samples were analyzed for PCDD/Fs as well as for dioxin-like polychlorinated biphenyls (dl PCBs) using the DR CALUX® method. The DR CALUX® bioassay method has been shown to be an effect-based toxicity screening analysis for all kinds of dioxin-like compounds, however, for confirmation it is recommended to obtain more specific congener analyses, which also allow examination of the PCDD/F congener patterns specific to different sources of pollution.

Ten samples in total were analyzed for content of individual PCDD/Fs and an extended list of PCB congeners by HRGC-HRMS at the accredited laboratory of the State Veterinary Institute in Prague, Czech Republic. Samples of bottom ash and sediment collected at the Ancing Road site and two ash samples from Kangshan were analyzed for specific PCDD/Fs and dl PCBs congener in MAS laboratory, Muenster, Germany, simultaneously with PBDD/Fs (by accredited method MAS_PA002, ISO/IEC 17025:2005 for PBDD/Fs). Some of the samples were also analyzed for content of hexachlorobutadiene (HCBD), pentachlorobenzene (PeCB) and hexachlorobenzene (HCB), in a Czech-certified laboratory (University of Chemistry and Technology in Prague, Department of Food Chemistry and Analysis). The analytes were extracted by a mixture of organic solvents hexane: dichloromethane (1:1). The extracts were cleaned by means of gel permeation chromatography (GPC). The identification and quantification of the analyte was conducted by gas chromatography coupled with tandem mass spectrometry detection in electron ionization mode.
### Table 1: Results of analysis for heavy metals and unintentionally produced POPs in samples from Taiwan in this study.

<table>
<thead>
<tr>
<th>ID</th>
<th>Locality</th>
<th>Matrix</th>
<th>Pb (mg/kg ww)</th>
<th>As (mg/kg ww)</th>
<th>Hg (mg/kg ww)</th>
<th>Zn (mg/kg ww)</th>
<th>Cu (mg/kg ww)</th>
<th>Ni (mg/kg ww)</th>
<th>Cr (mg/kg ww)</th>
<th>Cd (mg/kg ww)</th>
<th>PCDD/Fs (pg WHO-TEQ/g dw)</th>
<th>PCDD/Fs (ng/g dw)</th>
<th>PBDD/Fs (ng/g dw)</th>
<th>DR CALUX</th>
<th>HCB (pg/g dw)</th>
<th>HCB (pg/g dw)</th>
<th>PeCB (pg/g dw)</th>
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<tbody>
<tr>
<td>No. 1</td>
<td>Yan Chao</td>
<td>soil</td>
<td>69.19</td>
<td>&lt; LOD</td>
<td>&lt; LOD</td>
<td>101.13</td>
<td>42.37</td>
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<td>NA</td>
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<td>0.05*</td>
<td>0.05*</td>
<td>&lt;0.002*</td>
<td>&lt;0.212</td>
<td>0.225</td>
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<td>51.12</td>
<td>&lt; LOD</td>
<td>3,944</td>
<td>1,847</td>
<td>176</td>
<td>471</td>
<td>14.36</td>
<td>281</td>
<td>297.3</td>
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<td>Kangshan</td>
<td>ash</td>
<td>917.17</td>
<td>&lt; LOD</td>
<td>&lt; LOD</td>
<td>3,566</td>
<td>1,931</td>
<td>187.56</td>
<td>455.1</td>
<td>13.43</td>
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<td>&lt; LOD</td>
<td>4,167</td>
<td>1,951</td>
<td>163</td>
<td>655</td>
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<td>No. 15</td>
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<td>&lt; LOD</td>
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<td>273</td>
<td>43.71</td>
<td>52.56</td>
<td>97.32</td>
<td>&lt; LOD</td>
<td>8.17</td>
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<td>Fang Lyao</td>
<td>soil</td>
<td>40.17</td>
<td>8.88</td>
<td>&lt; LOD</td>
<td>155</td>
<td>41.78</td>
<td>&lt; LOD</td>
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<td>&lt; LOD</td>
<td>1.48</td>
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<tr>
<td>No. 17</td>
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<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>1.07</td>
<td>1.89</td>
<td>2.1</td>
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</table>

Notes: * Analysis of heavy metals by State Veterinary Institute, Prague by using atomic absorption spectrometry. Results are in mg/kg dry weight (dw). ww = levels of heavy metals were measured directly in wet weight by XRF. LOD = level of detection; LOQ = level of quantification.

* Levels in shrimp are per gram of fresh weight (fw); levels in eggs are per gram of fat.
For a quick understanding of the toxic characteristics of these samples, a handheld XRF analyzer (Thermo Scientific NITON® XL3t 700 XRF) was used to analyze heavy metals (lead, arsenic, mercury, zinc, copper, nickel, chromium, cadmium) content by Tainan Community University. Heavy metals were analyzed in soil and dust samples by XRF after their collection. Three samples (soil sample No.3 and fly ash sample No.4 and shrimps) were analyzed for selected heavy metals (mercury, cadmium, chromium, lead and arsenic) in the Czech laboratory of the State Veterinary Institute in Prague. Chemical analyses of heavy metals were performed by atomic absorption spectrometry (AAS). Sampling and analytical methods are described in the broader report10.

3. Results:

All results are summarized in Table 1.

PCDD/Fs prevailed in TEQ levels compared to dl PCBs in all samples except shrimps from the pond at Ancing Road, and dl PCBs contributed to TEQ levels by less than 15%. It was only in shrimps and eggs samples (Fang Lyao) that dl PCBs contributed to total TEQ by more than 40%. Among the samples fromsouth Taiwan, the highest content of PCDD/Fs + dl PCBs of 291 pg WHO-TEQ/g dw was measured in a fly ash from Yan Chao landfill site. The level measured in fly ash from the Mucha WI located close to Taipei was much higher and reached 860 pg WHO-TEQ/g dw.

PBDD/Fs were analyzed in two samples from Ancing Road and two samples of ash from Kangshan. The bottom ash from Ancing Road had 50.2 pg WHO-TEQ/g dw, while the sediment had 21.7 pg WHO-TEQ/g dw. The ash samples from Kangshan had higher levels of PBDD/Fs (58 and 61 pg WHO-TEQ/g dw) than Ancing Road samples. PBDD/Fs contributed significantly to the total dioxin toxicity of both analyzed samples from Ancing Road, as shown by DR CALUX results. DR CALUX® analysis for dioxin activity was conducted on six samples. Bottom ash from Ancing Road and dust from Kangshan showed high levels of dioxin activity, with the highest level found in the Kangshan sample taken at the foot of the landfill wall (see Table 1).

HCBD was analyzed in fly ash from Yan Chao, Mucha WI and eggs from Fang Lyao only. The level of 0.03 ng/g dw HCBD in Yan Chao was much lower than HCB and PeCB levels in the same sample. HCB and PeCB were comparably high in both Yan Chao and Mucha WI fly ash samples, exceeding 110 ng/g dw, while much lower levels were found in all other dust and soil samples, ranging from 0.04 to 0.59 ng/g dw. The highest HCB level of 0.59 ng/g dw was found in a soil sample from Yan Chao.

Heavy metals were analyzed in soil and dust samples by XRF while another two samples from Yan Chao were also analyzed by AAS in a laboratory. Measured levels of lead were comparable for both methods, while levels of mercury, chromium, and arsenic differed (see Table 1). The highest levels of lead were found in ash samples from all locations (in the range of 658 – 1109 mg/kg). The level in dust from Kangshan (1004 mg/kg) was comparable to the level in a stabilized fly ash sample from Yan Chao and was the highest lead level measured among the samples from Kangshan.

4. Discussion:

Studies in Taiwan have examined levels of unintentionally generated POPs, PCDD/Fs and dl PCBs, in WI residues since the 1990s11-13. More recent studies have also investigated polybrominated diphenyl ethers (PBDEs) and brominated dioxins and biphenyls13. Most recently the characteristics of PCDD/F content in fly ash discharged from MSWIs in Taiwan was summarized in a study from 201114. However, there is a lack of data on hexachlorobenzene (HCB), pentachlorobenzene (PeCB), and hexachlorobutadiene (HCBD) in WI residues from Taiwan, making the data in this report unique. Dioxin levels in fly ash from waste incinerators in Taiwan have decreased since the 1990s and ranged from 281 to 827 pg WHO-TEQ/g dw in this study. PCDD/Fs levels in fly ash from municipal waste incinerators in EU range from 0.2 to 23.9 ng I-TEQ/g dw, with most incinerators showing levels between 0.2 and 5.6 ng I-TEQ/g dw15. Similar levels were observed in studies from Denmark16 and the Czech Republic17. In China, fly ash from waste incinerators had levels ranging from 0.034 to 2.5 ng WHO-TEQ/g dw18.

The PCDD/Fs levels measured in this study (0.3-0.8 ng WHO-TEQ/g dw) fall within the range observed in MSWIs from other countries. The higher level of PCDD/Fs in fly ash samples from the Mucha incinerator could be explained by the different air pollution control system settings and the fact that the other fly ash samples were stabilized with cement and water. PBDD/Fs in fly ash have only been measured in a few studies from Taiwan, ranging from 0.00383 to 2.02 pg WHO-TEQ/g dw7,13, which is much lower than levels of PCDD/Fs and also lower than PBDD/Fs in bottom ash7. However, suspected bottom ash samples mixed either with fly ash or soil from Kangshan showed much higher levels of PBDD/Fs at 58 and 61 pg WHO-TEQ/g dw, comparable to those found in bottom ash from Ancing road. These samples were taken from weathered ashes at a fly ash burial site, where they may have become mixed with fly ash. PCDD/Fs levels in bottom ash samples from the Ancing Road site were
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found to be rather high compared to other sites in Taiwan, but within the range of measurements from two waste incinerators in 2010. Also, the level of PBDD/Fs of 50 pg WHO-TEQ/g dw was much higher compared to level of 6.3 pg TEQ/g dw measured in bottom ash from a waste incinerator in south Taiwan in the study published in 2010 but it was at the same level as the highest concentration of PBDD/Fs (52 pg TEQ/g dw) measured in bottom ash samples in another study published also in 2010. Higher levels of PBDD/Fs in bottom ash comparing to fly ash were also observed in waste incinerators from Japan.

Levels of HCB and PeCB in fly ash samples in this study within the ranges 116 – 139 ng/g dw and 111 – 153 ng/g dw respectively were comparable with highest levels measured in six WI fly ash samples from China which were 120 and 150 ng/g dw respectively but several times higher than maximum levels measured in MSWIs in Vietnam 30 and 27 ng/g dw respectively. However, levels of HCB in fly ash samples (<LOQ – 0.03 ng/g dw) were much lower in this study comparing to six fly ash samples from China.

The level of PCDD/Fs in free-range eggs from Fang Lyao (1.07 pg WHO-TEQ/g fat) is higher than the background level of caged eggs from Taiwan (0.274 pg WHO-TEQ/g lipid). It is also higher than levels of 0.533 and 0.469 pg WHO-TEQ/g lipid respectively measured in free-range chicken eggs from areas closer to mountains in the same study, but it is lower than maximum level of 4.29 pg WHO-TEQ/g lipid in that study.22 However, the concentrations of both PCDD/Fs and total WHO-TEQ level for PCDD/Fs plus d,l PCBs are below limit values set for eggs as food in the EU (2.5 and 5 pg WHO-TEQ/g lipid respectively).23 We can conclude that the PCDD/Fs level in free-range eggs from Fang Lyao do not exhibit levels as high as those at other places in southern Taiwan or globally, but they are more contaminated with PCDD/Fs and d,l PCBs than background samples from caged chicken eggs from Taiwan or other background samples from Southeast Asia. The contamination of eggs in Fang Lyao is possibly due to WI ash stored at a nearby landfill. PCDD/F congeners pattern in the eggs is similar like in soil samples from Fang Lyao as well as like bottom ash and sediment sample from Aencing road (see Figure 1).

Soil samples from Fang Lyao show PCDD/F levels 8 times higher than the average level for agricultural soils in Tainan region. However, levels in Yan Chao are lower and similar to the reference level. HCB, PeCB and HCBD levels in Taiwan soils are not available for comparison. Dust samples from Kangshan outside the landfill walls indicate potentially high contamination of the neighborhood. The global mean background soil HCB concentration was 0.68 ng/g dw. None of the soil samples in this study exceeded global background levels for HCB.

The heavy metal levels measured in this study were lower than the maximum levels found in a recent Chinese study for lead and mercury, but comparable with geometric mean in that study (1.420, 6.14 and 57.3 for lead, mercury and arsenic). Comparable levels in ash samples in our study for all these three heavy metals were found (see Table 1). The highest levels of lead in soils were found in samples from Yan Chao and Fang Lyao, accompanied by increased levels of zinc.
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5. Conclusions:
Increased levels of PCDD/Fs and dl PCBs were found in samples of dust, soil and/or eggs at two studied localities in the vicinity of operating or closed landfills which store fly ash from municipal waste incinerators and use bottom ash as a cover layer at these landfills (Kangshan and Fang Lyao). In one case (Yan Chao) increased levels of PCDD/Fs and dl PCBs were not observed either in soil or sediments in the vicinity of the landfill. Contamination of sediments in a pond next to dumped WI bottom ash reached significant levels of PCDD/Fs and PBDD/Fs at another studied site (Ancing road). This confirms findings in previous studies. Free-range eggs from Fang Lyao were more contaminated with PCDD/Fs than background samples from caged chicken eggs from Taiwan or other background samples from Southeast Asia. Contamination above the limit of quantification of dl PCBs was measured in samples of shrimps from Ancing road. These results also confirm that WI residues can be significant sources of contamination with unintentionally produced POPs. Their control also needs to be improved by setting stricter limits for POPs in wastes. PBDD/Fs should be added to the POPs regulated by the Stockholm Convention as they seem to contribute significantly to the overall dioxin toxicity of MSWIs residues and the environment surrounding sites where these residues are disposed of.

6. Acknowledgments:
The study was financially supported by the Government of Sweden through IPEN, the Global Greengrants Fund, and the Sigrid Rausing Trust.

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Fly ash generated by incinerators contains high concentrations of dioxins and must be properly treated. The sludge produced by sewage treatment plants is also a waste product that needs to be treated because it contains various harmful substances such as heavy metals, organic pollutants, and bacteria. Sulfur oxides and nitrogen compounds generated by heating sewage sludge can poison catalytic metals and occupy active sites, effectively inhibiting the formation of precursors and de novo synthesis and reducing PCDD/F concentrations. Therefore, using sludge as an inhibitor for low-temperature pyrolysis of fly ash can simultaneously treat both waste products and improve the destruction efficiency of PCDD/Fs in fly ash. This study aims to integrate water washing with low-temperature pyrolysis for co-processing of fly ash and sewage sludge to improve the destruction efficiency of PCDD/Fs in fly ash generated from MWIs. The results showed that the chloride content of fly ash was reduced from 16.48 ± 0.78 wt% to 10.45 ± 0.56 wt% after water washing. Experimental results obtained from the pyrolysis of fly ash with dry sewage sludge at 350°C for 5 minutes indicate that PCDD/F reduction efficiency based on mass concentration is over 99% and the reduction efficiency based on TEQ concentration is over 96%. These efficiencies were obtained when the mass ratio of washed fly ash (WFA) to dry sewage sludge (DSS) was controlled at 1.0 and the TEQ concentration was greatly reduced from 9.16 ± 0.01 ng I-TEQ/g-WFA to 0.11 ng I-TEQ/g-WFA with 5 minutes of reaction. Moreover, as the reaction time was extended to 10 minutes, the TEQ concentration was further reduced to 0.01 ng I-TEQ/g-WFA to meet the European End of Waste Criteria (20 pg TEQ/g). This study demonstrates that pyrolysis of washed fly ash with dry sewage sludge can greatly reduce the dioxin content in fly ash. Moreover, the optimal operating temperature is also explored. The approach developed in this study has the potential to simultaneously achieve the goals of pollution reduction and resource reuse.

References:
**THU-PM1-C5**  Emission of per- and polyfluoroalkyl substances from commercial facility recycling industrial waste for production of refuse derived paper and plastics densified fuel

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**Introduction:** Recycling of industrial wastes for production of refuse derived paper and plastics densified fuel (RPF) has great economic potential for energy recovery from waste materials and to reduce the amounts of waste materials incinerated or sent to landfill. Without any material and chemical recycling measures, it is an essential measure for managing a huge number of industrial wastes from social and economic activities. However, there is growing concern about the environmental emissions and potential human exposure to per-and polyfluoroalkyl substances (PFAS) from municipal and industrial waste disposal. PFAS are used in a wide range of consumer products, such as durable water repellent (DWR) clothing and food contact materials, and consequently, the discarded clothing and papers containing PFAS must be mixed into the industrial wastes. Nevertheless, there is a lack of studies on PFAS released during recycling of industrial wastes for RPF production. Here, we investigated the emission of PFASs in a commercial facility recycling industrial waste for RPF production.

**Materials and Methods:** In May and December 2022, we collected samples of RPF and shredded industrial waste before heat-molding for RPF production. Then we collected samples of outdoor and indoor air using active low-volume air samplers (LVAS) and passive air samplers (PAS) within XAD-2 resin. Using the LVAS method, the air samples were collected for 5 hours with a flow rate of 20 L/min, while the PAS method was used for one month to capture gaseous PFAS. In addition, we collected a sample of exhaust gas released from heat-molding machine for RPF production. 76 PFAS in the sample extracts were analyzed using LC-TOF-MS and GC-MS/MS.

**Results and Discussion:** PFAS were detected in all samples of outdoor and indoor air collected at the first sampling round in May 2022. The highest concentrations were observed for four fluorotelomer alcohols (4:2, 6:2, 8:2, and 10:2 FTOHs) and 6:2 fluorotelomer methacrylate (6:2 FTMAC) in the air sample collected near the heat-molding machine for RPF production, while the concentrations of FTOHs and FTMAC in the air samples collected near the shredding machine for industrial wastes and outside of the facility were one order of magnitude lower than those near the heat-molding machine. In addition, we found that concentrations of FTOHs and FTMAC in the sample of exhaust gas from the heat-molding machine was one order of magnitude higher than that collected near the heat-molding machine. Therefore, the main source of PFAS emission in the facility recycling industrial waste for RPF production were the heat-molding machine. Interestingly, the results obtained from analysis of PFAS in the samples of RPFs and shredded industrial wastes indicate that textile and paper wastes were the predominant wastes containing FTOHs and FTMAC. We assume that vaporization of PFAS can be promoted by heating shredded textile and paper wastes over 120 °C during RPF molding.

**Conclusion:** Results of the present study provided information related to emission of PFAS from recycling of industrial wastes for RPF production. Heating the shredded textile and paper wastes has been determined to be important factors contributing to the emissions of PFAS to the indoor environment. Special attention should be paid to the exhaust gas from the heat-molding machine for RPF production. This information obtained from the present study would be useful for planning indoor exposure avoidance and appropriate measures for emission control of PFAS released during recycling of industrial wastes for RPF production.

**Acknowledgments:** This research was supported by the Environment Research and Technology Development Fund [3-2102(1) (2): JPMERF20213002] of the Environmental Restoration and Conservation Agency (ERCA) of Japan.

**References:**
THU-PM1-D1  Occupational and residential exposure to micro-/nanoplastics and their decomposition products studied by gas chromatography x cyclic ion mobility-mass spectrometry

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Introduction: Humans are likely exposed to micro-/nanoplastics (MNPs) through inhalation, but few studies have attempted to measure airborne MNPs. This might be attributed, in part, to a paucity of analytical techniques that can identify and quantify MNPs without constraints related to their size. This presentation reports on the development of a novel (non)targeted screening (NTS) approach to identify and quantify MNPs in the indoor environment using pyrolysis gas chromatographic cyclic multiplexed with ion mobility mass spectrometry (pyr-GC×cIMS).

Materials and Methods: The cIMS enables the simultaneous measurement of m/z, collision cross section (CCS), and RT for all pyrolysis decomposition products as well as plastics additives sampled through thermal desorption. The method was applied to size-resolved particulate (56 nm-18µm) collected from two different indoor environments, viz. a laboratory space and a private residence. A variety of common plastic types were targeted, including polystyrene (PS), polyethylene (PE), polypropylene (PP), and polymethyl methacrylate (PMMA).

Results: The cIMS enables the simultaneous measurement of m/z, collision cross section (CCS), and RT for all pyrolysis decomposition products as well as plastics additives sampled through thermal desorption. The method was applied to size-resolved particulate (56 nm-18µm) collected from two different indoor environments, viz. a laboratory space and a private residence. A variety of common plastic types were targeted, including polystyrene (PS), polyethylene (PE), polypropylene (PP), and polymethyl methacrylate (PMMA). The results suggest that approximately 57-67% of airborne MNPs are characterized by particle diameters less than 2.5 µm. The NTS experiments also revealed the presence organophosphate esters whose abundance correlated with that of polyurethane (PU) (r = 0.85, p<0.05), which is consistent with their use as flame retardants in PU-based furniture and construction materials.

Discussion and Conclusion: The results of this study provide insight into the concentrations of MNPs in the indoor environment. The mean concentrations of MNP particles with diameters <2.5 µm in the two sampling locations, ranging from 16 – 27 µg/m3, exceed short term (24-hour) and long-term exposure guidelines suggested by the World Health Organization. This result, and the fact that approximately 50-60% of the PM2.5 present in the private residence are MNPs, underlines the critical need for further study of this route of exposure to MNPs and the (unknown) plastics additives carried with them.

Acknowledgments: The authors gratefully acknowledge funding from the Natural Sciences and Research Council of Canada (NSERC).
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Introduction: The global plastic pollution has crossed global boundaries of the safe operating space for humanity. The challenges were recognized by the United Nations Environment Assembly which initiated the development of a global plastic treaty. Plastics in their various applications contain a wide range of hazardous additives including several POPs listed in the Stockholm Convention, such as HBCD, HBB, PBDEs, PCB, SCCP, Dechlorane Plus, and UV-328 which although regulated are present in stocks (electronic products, vehicles, building materials, synthetic textiles). Environmental pollution from these POPs originates largely from the release from plastic or plastic pellets. In many developing countries, release is facilitated by open burning in end-of-life treatment and fires in dump sites. While a range of studies have been published on POPs in major product sectors, there is a lack of knowledge on POPs in plastic recyclates in developing countries with associated risks. Therefore, activities are conducted with support from the UNEP/GEF POPs global monitoring plan projects from February to June 2023 and the International Panel on Chemical Pollution (IPCP) was tasked to execute.

Materials and Methods: Plastic pellets and shreds from recycled plastic have been collected in Africa, Asia, and the Latin America and the Caribbean region from February to April 2023. Samples were sent to partnering laboratories of IPCP for analysis of selected POPs including NIES (Tsukuba, Japan), Fraunhofer Institute (Freising, Germany), IDAE-CSIC (Barcelona, Spain), and MTEC (Bangkok, Thailand). Selected recyclates used to produce food- or skin-contact products were screened by bioassays for genotoxicity, cell toxicity, or endocrine effects (Bio Detection Systems; The Netherlands).

Results: The project had major components (i) to develop a webinar series to strengthen capacities on POPs monitoring in plastics; (ii) to assess the state of knowledge and gaps on monitoring POPs and POP candidates in plastic in major use sectors, and (iii) to monitor selected POPs in plastic pellets and shredds of plastic recycled. The webinar series were delivered in April and May 2023 covering background information of environmental and human exposure to POPs in plastic, an introduction to individual POPs in plastic and on sampling, screening and analysis of POPs in plastics and recordings are available on the IPCP website. More than 300 recycled pellets and shreds samples used to produce new plastic products in developing countries of different polymers (e.g. PVC, ABS, HIPS, PS, EPS, XPS, HDPE, LDPE, PP, PA, PC) have been sampled with an initial assessment of plastic recycling in some of the countries. Gathered information revealed that some countries have a control regime for food contact materials, but some other countries did not. Analysis is currently conducted in the partner laboratories.

Discussion and Conclusion: In the coming years a considerable improvement of global plastic management is needed, including the control and elimination of POPs and other chemicals of concern in plastic and related recycling. More monitoring capacity and awareness on POPs in plastic is required to control, safeguard and promote recycling and as a support of the upcoming plastic treaty. The experience and approaches of controlling POPs in plastic including recycling can be valuable experiences for addressing other plastic additives of concern.

Acknowledgments: We acknowledge the support of the UNEP/GEF POPs GMP project.

References:
THU-PM1-D3 Monitoring of Toxicity of Plastic Recyclates by Bioassay Panel to Get an Integrated Assessment of Toxic Effects from the Complex Mixtures Present in Recycled Plastics

Peter A. Behnisch, Ludwig Gruber, Roland Weber

Introduction: Plastics in their various applications contain a wide range of hazardous additives or otherwise plastic related hazardous chemicals including several persistent organic pollutants (POPs) listed in the Stockholm Convention such as HBCD, HBB, PBDEs, PCB, SCCP, PFOS, PFOA, Dechlorane Plus, and UV-3281. In addition, a large number of non-intentionally added substances (NIAS) including degradation products, processing aids or impurities in plastic additives such as e.g. brominated dibenzofurans in PBDEs or HCB in certain pigments are present to various degree. Furthermore, the recycling of plastic are a source of NIAS which can include POPs if e.g. e-waste plastic is recycled into toys. While a range of studies have been published on POPs in major product sectors, there is a lack of knowledge on POPs in plastic recyclates in developing countries. Therefore, activities are conducted under the UNEP/GEF Global Monitoring Plan projects to collect plastic recyclates from selected developing countries from February to June 2023. In addition to chemical single compounds analysis to target POPs it was decided that also the screening of toxic effects by of selected plastic recyclates would be beneficial.

Materials and Methods: Plastic pellets and shreds from recycled plastic have been collected in Africa, Asia, and the GRULAC region from February to April 2023. For the screening of effects recycled pellets or shreds partly used for the production of food contact materials or skin contact materials (sandals) were selected. The migration experiments were conducted either by BDS or by Fraunhofer Institute IVV (Freising, Germany). Three different extraction/migration experiments with 0.5 g of plastic material have been used: Method A) with 10 ml tetrahydrofuran (THF) and two-times dropwise adding of 10 ml hexane (full extraction method); Method B with 50% ethanol/water mixture at 60°C for 3 days in an oven (similar to migration testing) and Method C with 20% ethanol/water at 40°C for one day (similar to skin contact migration testing). To the sample extracts were 50 µl DMSO (as keeper) added, evaporated under nitrogen gas until ca. 50 µl volume and screened by bioassays for cell death (Cytotox CALUX in tributyltin-EQ), genotoxicity DNA repair (p53 CALUX in Actinomycin-equivalents, EQ), endocrine disrupting chemical effects (female hormone activation by ER CALUX in estradiol-EQ and male hormone inhibition by anti-AR CALUX in flutamide-EQ) and PAH-like compounds (PAH CALUX in Benzo[a]pyrene-EQ) by standard operation procedures of BDS.

Results: The screening of the first four samples (LDPE, HDPE, PET and PVC based recyclates from Nigeria) reviled multiple in vitro toxicity effects including cell death, PAH-like, estrogen activity and anti-androgen toxic in the samples tested. None of the initial samples tested showed any genotoxic effect (no p53 DNA repair activation by p53 CALUX). In most cases the full extraction Method A resulted in higher toxicity compared to the two other applied migration tests (B and C). A particular high PAH toxicity in the PAH CALUX (45.000 ng BaP-EQ/g material) was detected in a black PVC sample from Nigeria in the extraction precipitation experiment indicating that carbon black containing PAHs might have been present.

Discussion and Conclusion: Bioassays are important tools to screen a wide range of toxic effects from migration experiments and from total extracts. The complex mixtures of hundreds of additives (IAS), unknown compounds (NIAS), their transformation products and thereof complex mixtures in recycled plastic require an integrated toxicity assessment which cannot be delivered by chemical analysis of target compounds as well as not by animal testing. While in this study a limited panel of 5 assays for different toxic endpoints has been applied, a wide range of further bioassays are available (such as for thyroid hormone disruption, dioxin-like activities or PFAS-like toxicities) and can be utilized in such in vitro toxicity screenings.

Acknowledgments: We acknowledge the support of the UNEP/GEF POPs GMP project.

References:
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THU-PM1-D4 Effect of Water Matrix on Triclosan Sorption on Two Types of Polyethylene

Introduction: The release of microplastics (MPs) has significantly risen in the aquatic environment and one of the most prevalent plastics found in the environment is polyethylene (PE) (Wu et al., 2016). Besides potential risks associated with ingesting MPs, they may act as vectors for organic contaminants (OCs) for marine organisms. In this study, as model compound, triclosan (TCS), which is widely used as an antimicrobial agent, is selected to understand the sorption behavior of OCs with microplastics in different water matrix.

Materials and Methods: High-denisty (HDPE, 0.935 g/cm³) and low-density polyethylene (LDPE, 0.919 g/cm³) were used in powdered form (dp=250-500 µm), and prior to use, sieved, washed, sonicated and dried at 30°C. TCS was analyzed spectrophotometrically at 279 nm. Isotherm experiments were conducted at 7 different solid/liquid ratios at predetermined teq at 9 ppm initial concentration, at pH 6. Water matrix effect was investigated using artificial seawater (SW) with salinity of 35 ppt and 52.8 mS/cm conductivity, artificial freshwater (FW) with zero salinity and 0.086 mS/cm conductivity, 5 ppm humic material solution (HM) with 0.010 mS/cm conductivity and distilled water (DI). All experiments were conducted as triplicates. When the concentration of HM in the MP-only control reactor exceeded 1%, the data was presented as control corrected.

Results: FTIR confirmed PE backbone, crystallinity (from DSC) were 49% and 25%, & pH_PZC ≈2.0 and 1.8 for HDPE and LDPE, respectively. Kinetic experiments yielded teq=24h for HDPE and 6h for LDPE. TCS sorption capacity of HDPE (7390µg/g) was higher than that of LDPE (5157µg/g). Sorption data fitted to Freundlich model showing a plausible fit, with Freundlich exponents (n_f) of TCS on HDPE (R²=0.998) 0.84 and 0.85 for LDPE (R² = 0.991). Lastly, order of TCS sorption for both HDPE and LDPE for tested water matrices were SW>FW=HM=DI. In all instances, TCS sorption on HDPE was higher (above 90%) than its sorption on LDPE (above 80%).

Discussion and Conclusion: Isotherm studies with both polymers showed close to linear sorption trend owing to high affinity of TCS on PE. During the experimental pH of 6, TCS (pKa= 8.1) is in its undissociated form. Yu & Bi (2016) state hydrophobic partitioning of undissociated phenolic species into organic matter to be linear. Besides, HDPE and LDPE surfaces are negatively charged beyond pH 2. We observed much less sorption when tests were conducted at pH 10, TCS being in phenolate form.

Acknowledgments: This study was funded by TUBITAK Project No: 220N044.

References:
THU-PM1-D5  Tetrachloroethane Sorption onto Pristine and Aged Polyethylene Type Microplastics

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Introduction: Low density polyethylene (LDPE) is extensively utilized and wasted as plastic bags. LDPE type microplastics undergo UV-aging causing physicochemical changes in the polymeric structure, in turn affecting sorption capacity and interaction with organics. The aim of the study was to comparatively evaluate sorption of 1,1,2,2-tetrachloroethane (PCA), an aliphatic chlorinated compound onto pristine and UV-aged LDPE.

Materials & Methods: LDPE (density = 0.919 g/cm3) was aged in a custom-made cabin containing UV-A lamps, at a UV dose of 2800 joule/cm2, at approximately 70°C. Aging was characterized by FTIR (via carbonyl index - CI calculation with SAUB method), DSC (for % crystallinity) and SEM imaging. Characteristics of pristine and aged LDPE were: T_melting=101.7°C and 103.8°C, crystallinity 24.7% and 24.9%, CI =0 and 0.22-1.40, respectively. Liquid-liquid extraction and analysis of PCA via GC-ECD were conducted according to USEPA Method 551.1 (1995). Sorption experiments were carried out in 40mL vials as triplicate, at 25°C ± 2°C, shaken at 200 rpm, having solid-liquid ratio of 25 g/L, with 250-500 µm size LDPE using DI water. Isotherms included a minimum of 10 concentrations. PCA-only, MP-only and DI-only controls also set-up, yielding no contamination or more than 5% analyte loss during sorption.

Results: UV-aging of LDPE was confirmed with FTIR, SEM and DSC. Kinetic experiments showed sorption of PCA onto LDPE to be very fast with teq=6 h. Kinetic data best fits pseudo-second order model (R2=0.996). Sorption isotherms yielded sorption capacity of 84.1±1.9 µg/g for pristine and 119.1±2.3 µg/g for aged LDPE (CI= 0.9). On average, pristine LDPE showed 26% less sorption, regardless of initial concentration. Isotherm data are fitted to five isotherm models and the best fitted model for both pristine (R2=0.994) and aged LDPE (R2=0.991) was Langmuir isotherm. LDPE-water sorption coefficient was more than double for aged when compared to pristine LDPE. Lastly, change of PCA sorption efficiency with LDPE at various stages of aging (CI ranging from zero to 1.4) showed that as aging increases, sorption of PCA shows a slowly decreasing yet increasing trend (Figure 1).

Discussion and Conclusions: A positive relationship between degree of aging and sorption of PCA was observed, as supported by other researchers with polyethylene (Bhagat et al., 2022, Li et al., 2022). Experimental sorption coefficients obtained in this study are on the same order of magnitude with those reported for other chlorinated aliphatics with HDPE (Uber et al., 2019). Our study revealed that formation of oxygen-containing functional groups with UV-aging (as indicated by an increase in CI) did not prevent, on the contrary contributed to PCA sorption. It should be emphasized that under natural conditions, pristine MPs are rare. Since aging of MPs increase with time, this creates a concern due to the increased risk of pollutants accumulating on MPs, potentially exacerbating the vector effect.

Figure 1. Change of PCA sorption as a function of aging and SEM images for pristine and aged LDPE.

Acknowledgments: This study was funded by TUBITAK Grant No: 220N044.

References
THU-PM2-A1  What's the F[luorine]? Contribution of Pharmaceuticals to Unknown Organofluorine Mixtures in U.S. Adult Serum

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Introduction: Organofluorines occur in human serum as complex mixtures of known and unidentified compounds. Human biomonitoring traditionally uses targeted analysis to measure the presence of known and quantifiable per- and polyfluoroalkyl substances (PFAS) in serum, yet characterization of exposure to and quantification of PFAS are limited by the availability of methods and analytical standards. Studies comparing extractable organofluorine (EOF) in serum to measured PFAS using organofluorine mass balance show that measurable PFAS only explain a fraction of EOF in human serum and that other sources of organofluorine may exist. The gap in fluorine mass balance has important implications for human biomonitoring because the total body burden of PFAS cannot be characterized and the chemical species that make up unidentified EOF are unknown. Many highly prescribed pharmaceuticals contain organofluorine (e.g., Lipitor, Prozac) and are prescribed with dosing regimens designed to maintain a therapeutic range of concentrations in serum. Therefore, we hypothesize organofluorine pharmaceuticals contribute to EOF in serum.

Materials and Methods: We use combustion ion chromatography to measure EOF in commercial serum from U.S. blood donors. We used LC-MS/MS to measure 44 targeted PFAS. Using fluorine mass balance, we assess differences in unexplained organofluorine (UOF) associated with pharmaceutical use and compare them with concentrations of organofluorine predicted based on the pharmacokinetic properties of each drug.

Results: Pharmacokinetic estimates of organofluorine attributable to pharmaceuticals ranged from 0.1 to 55.6 ng F/mL. Analysis of 44 target PFAS and EOF in samples of commercial serum (n = 20) shows the fraction of EOF not explained by S44 PFAS ranged from 15% to 86%. Self-reported use of organofluorine pharmaceuticals is associated with a 0.36 ng F/mL (95% CL:1.26 to 1.97) increase in UOF, on average, compared to those who report not taking organofluorine pharmaceuticals.

Discussion and Conclusion: Our study is the first to assess sources of UOF in U.S. serum and examine whether organofluorine pharmaceuticals contribute to EOF. Discrepancies between pharmacokinetic estimates and EOF may be partly explained by differences in analytical measurements. Future analyses using EOF should consider multiple extraction methods to include cations and zwitterions. Whether organofluorine pharmaceuticals are classified as PFAS depends on the definition of PFAS.

Acknowledgments: National Institute of Environmental Health Sciences R01 ES027813 (Webster PI). E.P. is supported by the National Institute of Environmental Health Sciences training grant (T32 ES014562).
Comparison of Exposure to PFOS from Diet and Personal Care Products with Concentrations in Blood Using a PBPK Model – Preliminary Results from the EuroMix Study as part of the EU project ONTOX

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Introduction: Per- and polyfluoroalkyl substances (PFAS) constitute a large group of compounds that are water, stain, and oil-repellent. Numerous sources contribute to the blood levels of PFASs in the European population. The main contributors for perfluorooctanoic acid (PFOS) are food/drinking water, house dust, consumer products and personal care products (PCPs). However, only a few studies have estimated the exposure from consumer products and PCPs, due to a lack of information. The purpose of the present work is to calculate the dietary and dermal external exposure to PFOS, estimate the aggregated internal exposure from diet and PCPs using a physiologically based pharmacokinetic (PBPK) model, and compare estimated and measured concentrations.

Materials and Methods: The EuroMix study (random selection n=60 of the 144 participants) was used for an external exposure estimate of PFOS from diet and personal care products (PCPs). For the study details see Husøy, Andreassen et al. (2019). Weighted dietary records and PCP diaries were used in combination with concentration data of PFOS in Norwegian foods and PCPs (EU/Canada/USA) in a probabilistic exposure assessment. The PBPK model (EFSA 2020) was further refined by incorporating a dermal exposure pathway, and modifications in the kidney and faecal excretion. The estimated internal PFOS concentrations were compared with measured PFOS concentrations in blood using the lower bound (LB, < LOD = 0) for the exposure and the PBPK modelling.

Results: The aggregated internal exposure using the PBPK model for PFOS shows that the major contributor is the diet for both males and females, and the estimated internal concentration attributed to diet is 10-100 times higher than from PCPs. The estimated aggregated internal exposure is higher than the measured exposure in blood for PFOS (Figure 1), where fish is the major contributor to the exposure. The concentration data for PFOS in foods have a high proportion of non-detects (73%) and sporadic 100 times high concentrations of PFOS in fish samples.

Discussion and Conclusion: Recent work on aggregated internal exposure to PFOA showed that PCPs and diet contributed in a similar range as the internal PFOA exposure for several women in EuroMix (Husøy et al in prep). On the contrary, PCPs were found to be a minor source for PFOS exposure, which is in line with the lack of association of PFOS with cosmetic use (Thepaut et al., 2021). The estimated external dose based on the LB concentrations in food needs further refinement before the validation of the PBPK model with the remaining EuroMix participants. This includes an extension of the concentration database with PFOS data in foods from other countries to improve the exposure estimates.

Acknowledgements: Funded by Horizon 2020 Research and Innovation program (Grant Agreement No 963845).

References:

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Figure 1: Cumulative probability of estimated serum concentration from food (LB), PCPs dermal (LB) and aggregated, and measured total PFOS concentration in serum.
Per- and Polyfluoroalkyl substances (PFAS) are a class of synthetic organic chemicals comprising thousands of compounds. Their chemical and thermal stability along with their hydrophobic and lipophobic properties has made them popular for use in a wide range of products (Buck et al., 2011; Glüge et al., 2020). PFAS were first detected in human serum in 2001 (Hansen et al., 2001). Since then, possible human health effects of PFAS have been highlighted by different regulators e.g. EFSA, which highlighted significant health effects of PFAS i.e. lowered immune response to vaccinations and impacted liver functions (EFSA CONTAM Panel, 2020).

More recently, PFAS have been identified in a wide range of products that come into contact with skin, i.e. cosmetics, hand sanitizers, water-repellent clothing and even school uniforms (Schultes et al., 2018; van der Veen et al., 2020; Whitehead et al., 2021; Xia et al., 2022). Furthermore, studies have found correlations between PFAS serum levels and the use of personal care products (Thépaut et al., 2021) and hand wipes (Kim et al., 2019; Poothong et al., 2019). Following this, an in vivo rat study and a human subject study have been conducted, both finding portions of their target PFAS able to permeate the skin (Abraham and Monien, 2022; Chen et al., 2022).

3D-human skin equivalent (3D-HSE) models are commercially available, multilayer dermal tissues that mimic the histological and physiological properties of normal human skin. These models make it possible to conduct dermal permeation studies without the use of human or animal subjects (Ragnarsdóttir et al., 2022). In this study, using 3D-HSE models we investigate the dermal permeation potential of 17 PFAS (10 perfluorocarboxylic acids (PFCAs) and 7 perfluorosulfonic acids (PFSAs)). We present the distribution of the PFAS after exposure and compare the fractions that permeated compared to the fraction retained within the skin. Finally, our results are compared to those of previously reported papers.

1. Introduction:
Per- and polyfluoroalkyl substances (PFAS) are a class of synthetic organic chemicals comprising thousands of compounds. Their chemical and thermal stability along with their hydrophobic and lipophobic properties has made them popular for use in a wide range of products. The tested chemicals and their properties have been previously reported (Harrad et al., 2019). In short, receptor fluid samples were extracted using a Chromabond™ PFAS (6 mL/300 mg, Chromabond) SPE cartridge followed by clean-up using EnviCarb™ SPE cartridges. Skin samples were homogenized in 2 mL MeOH using ATH followed by 5 min sonication and centrifugation. This was repeated twice more with aliquots of solvents collected and combined after each step. The samples were concentrated to ca 1 mL and PFAS were extracted using the same
protocol as RF samples. Cotton bud samples were extracted with basic MeOH by ultrasonication followed by a clean-up by passing through 0.2 mm syringe filters. Donor and receptor compartment washes were concentrated down using a gentle N2 flow followed by cleanup using 0.2 mm syringe filters or EnviCarb™ SPE cartridges respectively. Target PFAS were analysed on a Sciex Exion UPLC coupled to a Sciex 5600+ triple TOF MS operated in negative mode.

2.3. QA/QC
A "field" blank comprising of a skin tissue exposed to solvent only, otherwise treated as a sample was included in each sample batch (n = 4). No target compound was detected in concentrations above the limit of detection (LOD) in the blank samples. The efficiency of the experimental approach was investigated by calculating the mass balance. Results revealed generally good recoveries overall (76-113%, except for PFDS (41%) and PFOS (60%)) of the target compounds, indicating good recoveries and efficiency of the extraction method.

2.4. Data Analysis
Absorbed fraction and fraction retained within the skin were calculated based on concentrations detected in those compartments compared to the concentration of applied dose (equation 1a and 1b)

\[
f_{\text{absorbed}}(\%) = \frac{\text{PFAS in RF (ng)}}{\text{applied dose (ng)}} \times 100 \quad (1a)
\]

\[
f_{\text{retained}}(\%) = \frac{\text{PFAS in skin (ng)}}{\text{applied dose (ng)}} \times 100 \quad (1b)
\]

3. Results:
Results were grouped in three major compartments: the directly absorbed dose (cumulative concentrations in RF samples + receptor compartment wash), the skin (concentrations detected in the skin tissue after 36 h) and the unabsorbed dose (concentrations in skin surface wash and donor compartment wash after 36 h). Mass balance was calculated by comparing concentrations in each compartment. No target compound reached a steady state after 36-hour exposure. Table 1 lists the cumulative permeation of target PFAS as well as the PFAS detected in skin cells at the end of exposure. For our target PFCAs and PFSAs, cumulative permeation decreased with increasing carbon chain length.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cumulative penetration (ng/cm²)</th>
<th>Cumulative penetration (%)</th>
<th>Concentration in skin (ng/cm²)</th>
<th>Concentration in skin (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perfluoropentanoic acid (PFPeA, C = 5)</td>
<td>278.46</td>
<td>55.69</td>
<td>99.38</td>
<td>19.88</td>
</tr>
<tr>
<td>Perfluorohexanoic acid (PFHxA, C = 6)</td>
<td>178.46</td>
<td>35.69</td>
<td>85.00</td>
<td>17.00</td>
</tr>
<tr>
<td>Perfluorohexanoic acid (PFHxA, C = 7)</td>
<td>127.04</td>
<td>25.41</td>
<td>159.09</td>
<td>31.82</td>
</tr>
<tr>
<td>Perfluorooctanoic acid (PFOA, C = 8)</td>
<td>58.93</td>
<td>11.79</td>
<td>191.36</td>
<td>38.27</td>
</tr>
<tr>
<td>Perfluorononanoic acid (PFNA, C = 9)</td>
<td>3.57a</td>
<td>0.86</td>
<td>228.31</td>
<td>45.66</td>
</tr>
<tr>
<td>Perfluorodecanoic acid (PFDA, C = 10)</td>
<td>0.38a</td>
<td>0.08</td>
<td>303.61</td>
<td>60.72</td>
</tr>
<tr>
<td>Perfluoroundecanoic acid (PFUnDA, C = 11)</td>
<td>0.11a</td>
<td>0.02</td>
<td>332.32</td>
<td>66.46</td>
</tr>
</tbody>
</table>
The cumulative permeation of target PFCAs was highest for PFPeA with more than half (55.7%) of the applied dose being absorbed after 36 h. For PFSAs, PFBS had the highest permeation (48.7%), slightly lower compared to the PFPeA yet still substantial. Figure 1 depicts the association of cumulative permeation and carbon (C) chain length (1a) as well as logKOW (1b) (Chemspider, 2023; Wang et al., 2011). The cumulative permeation then decreased for both PFCAs and PFSAs with increasing C chain length. Negligible permeation was detected for C ≥ 9 PFCAs and C ≥ 8 PFSAs after 24 h and thus no further time-points were collected past 24 h for those compounds. In general, PFCAs had a higher cumulative permeation after 36 h compared to PFSAs. The PFAS concentration within the skin increased with increasing C chain length with the concentrations peaking for PFUnDA (C = 11) and PFNS (C = 9) in the skin. The skin concentration then decreased for higher C-chain compounds. The reason for the lower skin retention of the shorter chain PFAS is due to their ability to permeate through the skin and enter the RF.
AFTERNOON BREAKOUT SESSIONS II

THURSDAY 14 SEPTEMBER 2023

15:40 - 17:00

Per- and Polyfluorinated Substances (PFAS): Occurrence and Exposure
T.Groffen & P.Leonards

THU-PM2-A3  Evaluating Dermal Bioavailability of Perfluoroalkyl substances using in vitro 3D-Human Skin Equivalent Models

Figure 1: Association of cumulative permeation of PFAS after 36 hours and CF chain length (a) and logKOW (b) Error bars represent one standard deviation (n = 3). Included are values for compounds with CF £10 for both PFCAs and PFSA.

It seems that the functional groups play a role in permeation as PFCAs generally have a higher permeated fraction compared to the PFSA with the same C chain length. Furthermore, the physicochemical properties, e.g. logKOW seem to play a role in permeation with a clear negative correlation between logKOW and permeation for both PFCAs (r = -0.95; p = 0.0008) and PFSA (r = -0.99; p = 4.8 x 10^-5). However, one PFSA, PFPeS, did not follow this trend and had a higher cumulative permeation (40.4%, KOW = 4.36) compared to the PFCA with the log KOW values below (PFHxA, 35.7%, log KOW = 4.06) after 36 h.

4. Discussion:
The compounds with highest permeated fraction (C £ 7 PFCA and C £ 6 PFSA) fall under or very close to the Lipinski’s criteria of logKOW < 5 and molecular weight < 500 Da. This criteria was developed for the evaluation of drug delivery, contaminants and thus, we still see a fraction absorbed of compounds that do not fall under these rules while it decreases with the increasing MW and logKOW (Lipinski et al., 2001). A study by Franko et al. investigated the dermal permeation potential of PFOA using both human full thickness skin and epidermis. They saw very similar permeation (24%) for both skin models after 24 h with an additional 45% and 23% of the applied dose found within the skin models. Our findings show a lower permeation of PFOA through the skin (11.8%) but a similar fraction detected within the skin models (38.3%) and the full thickness skin. There are some differences between the present study and the previous study, the time of exposure 36 h vs 24 h (Franko et al.) as well as the dosing solution concentrations 500 ng compared to 0.5 mg (Franko et al.) which could explain the differences in our findings. To support this, Franko et al report significant dose-response increases in serum levels of PFOA in mice following dermal exposure to different concentrations of PFOA (Franko et al., 2012).

Higher C chain PFAS were able to enter the skin cells, and while a steady state was not reached for any target compound after 36 h it is less likely these high C chain PFAS will be able to reach the bloodstream (Ragnarsdóttir et al., 2022). However, a study on one human subject exposed to mass labelled 13C4-PFOA mixed into sunscreen revealed that the adsorption was very slow,
with plasma levels reaching a maximum of 132 ng/L, 22 days after application of a single 110 mg dose (Abraham and Monien, 2022). Furthermore, a study on rats found that the peak times of PFAS in the bloodstream after dermal exposure (8-72h) were significantly longer than what is seen after oral exposure (1-24 h), suggesting that dermal permeation of PFAS, while slower could exert a substantial contribution to the total body burden (Chen et al., 2022). Keeping this in mind, it could be possible for the fraction of the PFAS still detected in the skin after 36h to slowly cross the skin barrier and enter the bloodstream.

5. Conclusions:
This study provides information on the permeation potential of PFAS. Substantial permeation (> 5%) was seen for C £ 8 PFCAs and C £ 7 PFSAs. The shortest C chain compounds examined, PFPeA (55.6%) and PFBS (48.7%) had the highest permeated fraction of PFCAs and PFSAs respectively. A decrease was then seen in the permeation with increasing CF chain length. These findings suggest that dermal exposure to PFAS is not only possible but could contribute substantially to the total human body burdens. Further research is needed to calculate the lag time, permeation coefficient and flux of these compounds and other PFAS to better evaluate the implications for human exposure.

6. Acknowledgments:
This project has received funding from the European Union’s Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 860665 (PERFORCE3 Innovative Training Network).

7. References:
THU-PM2-A3 Evaluating Dermal Bioavailability of Perfluoroalkyl substances using in vitro 3D-Human Skin Equivalent Models

Per- and polyfluorinated substances (PFAS): Occurrence and Exposure

T. Groffen & P. Leonards

THU-PM2-A4  Investigation of PFAS in water repellents on the Japanese market

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1. Introduction:
Per- and polyfluoroalkyl substances (PFAS) have received worldwide attention because of their environmental persistence and toxicity. The Conference of the Parties of the Stockholm Convention on Persistent Organic Pollutants (POPs) listed PFOA and PFOA-related compounds in Annex A in May 2019. Per- and polyfluoroalkyl substances have been widely used in industrial and household products for almost 70 years[1]. Previous studies showed that water repellents having been purchased in Norway and Germany contained PFAS including PFOA-related compounds[2, 3]. Recent studies also showed that fluorotelomer-based polymers (FTPs) in water-repellent products were degraded to PFAS including PFOA-related compounds in the environment[4, 5]. In this study, we determined the concentrations of individual PFAS and extractable organic fluorine (EOF) in water repellents by LC-MS/MS and combustion ion chromatography (CIC), respectively. The mass balance analysis was useful to provide information on the extent of unknown fluorinated chemicals in water repellents.

2. Materials and Methods:
Twenty-eight and twenty-six samples of water repellents sold in 2011 and 2021, respectively, were collected in Japan. A two-hundred milligrams of a water-repellent sample was extracted in 10 mL methanol by ultrasonication for 30 min. The concentrations of target PFAS in the extracts were determined by LC-MS/MS and GC-MS/MS. The targets for LC-MS/MS and GC-MS/MS analysis were 72 PFAS including 19 PFOA-related compounds: 5 PFSAs (perfluoroalkyl sulfonic acids), 13 PFCAs (perfluoroalkyl carboxylic acids), 3 PFESAs (perfluoroalkyl-ether sulfonic acids), 5 PFECAs (perfluoroalkylether carboxylic acids), 3 FTSAs (fluorotelomer sulfonic acids), 3 FTCAs (fluorotelomer carboxylic acids), 3 FTUCAs (fluorotelomer unsaturated carboxylic acids), 2 PAPs (polyfluorinated phosphate esters), 3 diPAPs (polyfluorinated phosphate diesters), 2 FASAAs (perfluorooalkane sulfonamido acetic acids), 6 FASAs (perfluorooalkane sulfonamides), 3 FASEs (perfluorooalkane sulfonamideethanols), 3 FTABs (fluorotelomer sulfonamide betaines), 2 FTBs (fluorotelomer betaines), 3 FTOs (fluorotelomer olefins), 4 FTCr (fluorotelomer carboxylic acids), 3 FTACr (fluorotelomer acrylates) and 3 FTMAcr (fluorotelomer methacrylates). The concentrations of extractable organic fluorine (EOF) in the products were also determined by combustion ion chromatography (CIC). In addition, 1 mL of 1 M sodium hydroxide solutions were added to 5 mL of the extracts and combined extracts were reacted at 50°C for 24 hours[6], followed by individual PFAS analysis in order to examine PFAS degraded from polymers in the samples.

3. Results and Discussion:
3.1 Concentrations of individual PFAS in water repellents
Twenty-eight and twenty-six samples of water repellents sold in 2011 and 2021, respectively, were subjected to LC-MS/MS and GC-MS/MS analysis. Concentrations of individual PFAS in these samples are shown in Figure 1. The compounds mainly detected in the samples were 8:2FTOH, 10:2FTOH and 12:2FTOH, which correspond to PFOA-related compounds. PFOA-related compounds were detected in 24 of 54 products, and their concentrations were up to 100 mg/kg. In previous studies, 8:2FTOH was mainly detected in water repellents at up to 720 mg/kg[2, 3]. In addition to FTOHs, fluorotelomer olefines (8:2FTO, 10:2FTO), fluorotelomer iodides (8:2FTI, 10:2FTI), fluorotelomer acrylates (8:2FTAc, 10:2FTAc) and fluorotelomer methacyrates (8:2FTMAc, 10:2FTMAc), which also correspond to PFOA-related compounds, were detected in this study. Although these PFAS were not analyzed in previous studies of water repellents[2, 3], FTAc and FTMAc could be produced by backbone cleavages of fluorotelomer-based polymers (FTPs)[5], and FTI is a precursor in the synthesis of FTOH[7]. The technical guidelines of the Basel Convention determined low POPs content (LPC) for PFOA-related compounds as 50 mg/kg[8]. In this study, the concentrations of PFOA-related compounds were higher than LPC (50 mg/kg) in 5 of 28 products in 2011, whereas the concentrations exceeded LPC only in 1 of 26 products in 2021. For PFAS other than PFOA-related compounds, 6:2FTOH was detected in more than half of the products in 2021. The concentrations of PFOA-related compounds decreased and those of other PFAS increased from 2011 to 2021, indicating the effect on the listing of PFOA-related compounds as POPs in 2019. In a recent study, 8:2FTOH and 10:2FTOH were not detected in anti-fog sprays, whereas 6:2FTOH was detected at 3.4–11000 mg/L[9], possibly reflecting the shift among industries from C8 to C6 chain lengths[10].
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3.2 Concentrations of EOF in water repellents

Fifty-four samples of water repellents were subjected to CIC analysis. Concentrations of extractable organic fluorine (EOF) in these samples are shown in Figure 2. Extractable organic fluorine was detected in all of 32 products with fluorine-related descriptions on the ingredient labels at 32–8400 mg-F/kg, and detected in 8 of 22 products without fluorine-related description at 65–4600 mg-F/kg. The concentrations of EOF have no statistical difference between 2011 and 2021. Although the concentrations of individual PFAS were up to 72 mg-F/kg, the concentrations of EOF were up to 8400 mg-F/kg. The concentrations of EOF were up to 1200-fold higher than those of individual PFAS. In a recent study, concentrations of EOF measured by CIC were 190–21000 mg/L in anti-fog sprays, which are the same levels of concentrations in this study[9]. In addition, concentrations of individual PFAS in water-repellent products (food packages) were 0.024–2.2 ng-F/cm2, whereas those of EOF measured by CIC were 220–490 ng-F/cm2 in a recent study[10]; the concentrations of EOF were more than two orders of magnitude higher than those of individual PFAS. In this study, trace amounts of FTOH were detected in the water repellents at up to 68 mg-F/kg, compared to the high concentrations of EOF. Because FTOH is used as a raw material in the manufacturing of the fluorotelomer-based polymer (FTP)[2, 4], the main component in the water repellents could be the FTP. Although the FTP is difficult to detect in individual PFAS analysis, the CIC analysis enable us to determine the concentrations of organic fluorine which derives from FTP as EOF.

Figure 1: Concentrations of individual PFAS in water repellents. The samples with fluorine-related statements on the ingredient labels are marked with red rectangles.

Figure 2: Concentrations of EOF (extractable organic fluorine) in water repellents. The samples with fluorine-related statements on the ingredient labels are marked with red rectangles.
3.3 Concentrations of PFOA-related compounds after hydrolysis

Sodium hydroxide solutions were added to the extracts of water repellent samples and the extracts were hydrolyzed, followed by the analysis of individual PFAS in order to examine the degradations of PFAS from polymers. Concentrations of PFOA-related compounds before and after hydrolysis are shown in Figure 3. Some of PFAS mainly detected after hydrolysis were 8:2FTOH and 2-[(methylperfluoro-1-butanesulfonamido)ethanol (N-MeFBSE) were also detected after hydrolysis at up to 3700 and 7900 mg/kg, respectively. The concentrations of PFOA-related compounds, especially 8:2FTOH and 10:2FTOH, increased in 23 of 54 products by up to 3600 mg/kg from pre- to post-hydrolysis; their concentrations statistically increased after hydrolysis (Welch’s t-test: *p<0.01). The results suggest that PFOA-related compounds are degraded from FTPs in water repellents. In a recent study, concentrations of 6:2FTOH, 8:2FTOH and 10:2FTOH were increased by up to 71, 890 and 310 mg/kg, respectively by hydrolysis of textile samples[6], which are comparable to our study. A recent study also showed that the weathering of FTP-based outdoor clothes, which included the exposure to UV radiation, humidity, and temperature for 300 hours, resulted in increasing the concentrations of individual PFCA and FTOH including PFOA and PFOA-related compounds by up to 180 and 460 µg/m², respectively[4]. According to these results, fluorotelomer-based polymers (FTPs) in water repellents could be degraded to PFAS including PFOA-related compounds in the environment. The polymers that can be degraded to PFOA-related compounds are also defined as PFOA-related compounds in the Stockholm Convention[11]. Although the analysis of these polymers themselves is difficult only by individual PFAS analysis, the evaluations of PFOA-related polymers are important for the managements of products and wastes containing PFOA-related compounds. In this study, the concentrations of PFOA-related compounds were lower before hydrolysis but higher after hydrolysis than LPC in 10 of 54 products. The evaluations of PFOA-related compounds degraded from polymers are therefore needed in addition to the analysis of products before hydrolysis.

![Figure 3: Concentrations of PFOA-related compounds in water repellents before and after hydrolysis. (**: *p<0.01)](image-url)

3.4 Mass balance analysis of PFAS in water repellents

Extractable organic fluorine (EOF) was detected in 40 of 54 products by CIC, and contributions of individual PFAS to EOF in these samples are shown in Figure 4. High percentages of EOF are contributed by individual PFAS after hydrolysis in some products. In 7 products in 2011 (#12–16, #23, #25) and 2 products in 2021 (#35, #22), >80% of EOF were contributed by 8:2FTOH, 10:2FTOH and 12:2FTOH, which were PFOA-related compounds degraded from FTPs. In addition, N-MeFBSE, which is a PFAS with C4 chain, contributed to around 100% of EOF after hydrolysis in 2 products in 2021 (#12, #36), suggesting the use of polymers with shorter sidechains[11]. On the other hand, high percentages of EOF still remain unknown in most products. In 3 products (#01, #02 in 2011 and #02 in 2021) described as "urethane resins" in the labels, the concentrations of N-MeFBSE increased by >1100 mg/kg after hydrolysis. However, 50–70% of EOF still remain unknown. Although 20–40% of EOF in 6 products in 2011 (#07–11, #26) were contributed by individual PFAS before hydrolysis, remaining EOF (60–80%) were unidentified even after hydrolysis.
Per- and Polyfluorinated Substances (PFAS): Occurrence and Exposure
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These results suggest the occurrences of polymers hard to hydrolyze and unknown fluorinated compounds in water repellents. A recent study showed that some of the PFAS mainly detected in anti-fog sprays were 6:2 fluorotelomer ethoxylates (6:2FTEOs) and 6:2-6:2 fluorotelomer ether[9], which are not analyzed in this study. In addition, a variety of alternative PFAS including polymers, such as perfluoropolyethers (PFPEs), perfluoroalkyl trialkoxysilanes, polyperfluoroethoxymethoxy difluoroethyl PEG phosphate, and short-chain perfluoroalkyl alcohols such as 3:1FTOH and 5:1FTOH, have been used in consumer products[11, 12]. Further studies are needed to characterize the unidentified EOF in water repellents.

Figure 4: Contributions of individual PFAS to EOF. ‘*’ above selected bars shows that the concentrations of individual PFAS are higher than those of EOF. The samples with fluorine-related statements on the ingredient labels are marked with red rectangles.

4. Acknowledgments:
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Introduction:
Food ingestion has been established as an important human exposure route to many environmental contaminants. However, information regarding dietary exposure to organophosphate esters (OPEs) remains limited and has not hitherto been investigated for the UK population.

Materials and Methods:
This study measured concentrations of eight OPEs in 393 food samples divided into 15 food groups collected from Birmingham, UK. Samples were extracted, purified, and analysed using validated in-house techniques based on GC-MS.

Results:
Each of the eight OPEs targeted: tris(2-chloroethyl) phosphate (TCEP), tris(2-chloroisopropyl) phosphate (TCIPP), tris(1,3-dichloro-2-propyl) phosphate (TDCIPP), 2-ethylhexyl-diphenyl phosphate (EHDPP), triphenyl phosphate (TPHP), tri-n-butyl phosphate (TnBP), tris(2-butoxyethyl) phosphate (TBOEP), and tri-m-tolyl phosphate (TMTP), were detected above the limit of quantification (LOQ) in at least one of the food groups analysed. Concentrations were highest (mean $\Sigma 8$OPEs = 18.4 ng/g wet weight (ww)) in milk and milk products, followed by those in cereal and cereal products (mean $\Sigma 8$OPEs = 15.9 ng/g ww), with concentrations lowest in chicken’s eggs (mean $\Sigma 8$OPEs = 1.61 ng/g ww). Interestingly, concentrations in animal-derived foods (mean $\Sigma 8$OPEs = 44.2 ng/g ww) were statistically indistinguishable ($p > 0.05$) from those in either plant-derived foods (mean $\Sigma 8$OPEs = 36.8 ng/g ww) or industrially-processed foods (mean $\Sigma 8$OPEs = 32.1 ng/g ww). TBOEP, EHDPP and TPHP were the dominant OPEs detected. Estimated daily dietary intakes (EDIs) of $\Sigma 8$OPEs under mean and high-end exposure scenarios for the four age groups considered were: toddlers (420 and 1547) $>$ children (155 and 836) $>$ elderly (74.3 and 377) $>$ adults ($62.3$ and $278$) ng/kg bw/day respectively. Baby food contributed 39% of $\Sigma 8$OPEs exposure for toddlers, with non-alcoholic beverages contributing 27% of exposure for children, while cereal and cereal products (25%) and fruits (22%) were the main contributors for adults and the elderly.

Discussion and Conclusion:
The concentrations of OPEs in UK food stuffs were generally of the same order of magnitude as those reported for other countries and our estimates of dietary exposure were well below the corresponding health-based limit values.
THU-PM2-B2 Investigation of Tris (1-chloro-2-propyl) phosphate (TCIPP) Biotransformation in Urine Samples from an Occupationally Exposed Population

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Introduction: Tris (1-chloro-2-propyl) phosphate (TCIPP) is one of the major organophosphate flame retardants (OPFRs) present in the indoor and outdoor environment [1]. Humans can be exposed via oral intake through the dust or the diet or via inhalation and TCIPP and its metabolites have been detected in various human matrices [2,3]. TCIPP is classified as a suspected carcinogen by the WHO [4] and in human, epidemiological studies show associations between TCIPP exposure and allergies [5,6]. Knowledge of biotransformation pathways is important to elucidate potential bioavailability and toxicity of TCIPP and to identify potentially useful biomarkers.

Bis (1-chloro-2-propyl) hydrogen phosphate (BCIPP) and 1-hydroxy-2-propyl bis (1-chloro-2-propyl) phosphate (BCIPHIPP) have already been established as TCIPP metabolites in urine in previous studies [7,8]. In addition, recent in vitro experiments with human liver microsomes revealed carboxyethyl bis (1-chloro-2-propyl) phosphate (TCIPP-M1) and 1-chloro-3-hydroxyprop-2-yl bis (1-chloropropan-2-yl) phosphate (TCIPP-M3) as possible metabolites while bis (1-chloropropan-2-yl) (1-oxopropan-2-yl) phosphate (TCIPP-M2) was identified as an intermediate product [9]. These metabolites have not yet been reported in vivo. Since people occupationally exposed to TCIPP might have a higher intake of this compound, the aim of this study was to determine in vivo biotransformation of TCIPP in urine from workers.

Materials and Methods: Urine samples from workers (n=65) exposed to TCIPP were obtained and deconjugated before undergoing solid phase extraction. Extracts were analyzed using liquid chromatography coupled to a triple quadrupole mass spectrometer (LC-QQQ-MS/MS) for the target analysis of BCIPP and BCIPHIPP. The samples that showed the highest BCIPP and BCIPHIPP concentrations in the target analysis were injected in a liquid chromatograph coupled to a quadrupole-time-of-flight mass spectrometer (LC-Q-TOF-MS) for untargeted analysis. A suspect screening approach using a suspect list with in silico predicted metabolites by Meteor Nexus v.3.1.0 was employed to identify the TCIPP metabolites.

Results: 14 samples were selected for untargeted analysis. Suspect screening showed BCIPHIPP in all samples. TCIPP-M3 was also detected in all samples, while TCIPP-M1 was detected in 10 samples. Interestingly, BCIPP was only detected in 2 samples during suspect screening. TCIPP-M2 could not be detected in the in vivo samples, probably because this aldehyde will be rapidly converted into other metabolites in an in vivo situation.

Semi-quantification based on the area ratios of metabolite to the internal standard showed that BCIPHIPP was the major metabolite followed by TCIPP-M3 and TCIPP-M1, with BCIPHIPP showing area ratios 34 and 65 times higher than TCIPP-M3 and TCIPP-M1, respectively. BCIPP was formed to the least extent, in the two samples in which BCIPP was detected it showed area ratios 3500 times lower than BCIPHIPP, 100 times lower than TCIPP-M3 and 60 times lower than TCIPP-M1.

Discussion and Conclusion: These in vivo results confirm results from in vitro experiments revealing TCIPP-M1 and TCIPP-M3 as TCIPP metabolites in human urine. Currently, BCIPP and BCIPHIPP are used as biomarkers in urine to determine TCIPP exposure. However, TCIPP-M1 and TCIPP-M3 showed higher detection frequencies and higher area ratios of metabolite to the internal standard than BCIPP. Therefore, in the future, these two metabolites could be implemented as new biomarkers in targeted methods to more accurately determine TCIPP exposure.

Acknowledgments: We would like to thank the workers who voluntarily donated their urine to this project.

This work was supported by the Interuniversity Special Research Fund from Flanders (IBOF Grant BOFIBO2021001102) and the Exposome Centre of Excellence of the University of Antwerp.

References:
THU-PM2-B3  In Vitro Biotransformation and Evaluation of Biotransformation Products of Chlorinated Paraffins by Rat Liver Sub-Cellular Fractions

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Introduction: Because of persistence, bioaccumulation and toxicity, short-chain chlorinated paraffins (SCCPs, C10-13) have been listed as persistent organic pollutants since 2017 and are banned in many countries. However, little is known about medium-chain CPs (MCCPs, C14-17) and long-chain CPs (LCCPs, C>17). As a result, they are still used as alternatives for SCCPs and quantities produced worldwide exceed 2 million tonnes/year (van Mourik et al., 2016). In vivo studies of CP metabolism have been reported since 1980s and have revealed liver as the main organ that metabolizes CPs (Darnerud, 1984). Only recently, studies attempt to identify CP metabolites using in vitro models (He et al., 2021). However, such studies are limited because of the complexity of CP congeners and the lack of relevant analytical standards. This study uses rat liver S9 and single CP congeners to 1) investigate the relative extent of in vitro biotransformation of CPs, focusing on Phase 1 metabolism by different liver cytochrome P450 enzymes, CP chain lengths and chlorine positions, and 2) evaluate the biotransformation products formed. The biotransformation information gained in this study will be finally used to synthesize reference standards of the main biotransformation products.

Materials and Methods: Incubation experiments were performed using rat liver S9 (1 mg/mL), CP (1 µM) and NADPH (1 mM) in Tris-HCl buffer (100 mM, pH 7.4) at 37 °C. The reaction was quenched by an equal volume of ice-cold methanol at desired reaction times. 20 µL internal standard was added and the samples were centrifuged at 5000 rpm for 15 min. The supernatant was analyzed immediately by HPLC-qTOF-HRMS. Three different S9 mixtures were tested: uninduced rat S9, Aroclor 1254- and PB&BNF-induced S9. Ten different CP congeners ranging from C11 to C18 were tested.

Results: The CP degradation followed 1st-order kinetics. Compared with uninduced rat S9, Aroclor 1254- and PB&BNF-induced S9 increased the CP biotransformation rates by 57.6% and 272%, respectively. The longer the CP chain lengths, the slower the biotransformation. The chlorine positions markedly influenced the species of metabolites formed. In total, two mono-hydroxylated and one di-hydroxylated products, one carbonyl product and one carboxylic acid were identified.

Discussion and Conclusion: This study gives evidence for the P450-dependent in vitro biotransformation of CPs. This is in accordance to previous observations that CYP inhibitors produce inhibitory effects on CO2 formation from CPs in mice (Darnerud, 1984). The relatively low degradation rate of longer chain CPs may be related to their higher Log Kow values (Bettina et al., 2011). Concerning the uninduced liver sub-cellular fractions, we determined a half-life for SCCP of 1.36 h in rat S9. This contradicts the half-life (22-60 years) measured in uninduced rat liver microsomes by Dong et al., (2019). These long CP half-life times could be explained by the CP concentrations used in the incubation which exceeded CP solubility in aqueous reaction mixtures. No other in vitro rat models are available for comparison. However, concerning human liver microsomes, He et al., (2021) also found fast CP biotransformation compared with Dong et al., (2019)’s results.

Acknowledgments: We acknowledge funding from the European Union’s Horizon 2022 research and innovation programme under the Marie Sklodowska-Curie grant agreement REVAMP project No. 956374.

References:

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**Introduction:** Emission of PCDD/Fs from stationary sources has caused much attention worldwide. Among them, secondary copper sludge smelting (SCS) processes are especially considered as important sources of PCDD/Fs owing to the catalytic effects caused by copper on PCDD/Fs formation (Cieplik et al., 2003; Wang et al., 2003). In addition to PCDD/Fs, other chlorinated aromatic compounds such as polychlorinated biphenyl (PCBs), polychlorinated naphthalene (PCNs), and chlorobenzene (CBz) are regarded as the persistent organic compounds which also cause adverse effects on human health and environment. Therefore, investigation on the formation potential of chlorinated aromatic compounds including PCDD/Fs, PCBs, PCNs and CBzs provides essential information on the emission characteristics of hazardous air pollutants from SCS plants.

**Materials and Methods:** The air pollution control devices (APCDs) adopted and the sampling points of the SCS plant investigated are shown in Figure 1. All the flue gas samples were collected following the NIEA A807.75C and the simultaneous clean up method developed by our group previously. After that, samples were analyzed with HRGC/HRMS.

![Figure 1 APCDs adopted and sampling points of the SCS plant investigated (solid star represents flue gas sample; hollow star represents ash sample).](image1)

**Results and discussion:** The results indicate that the mass concentrations of PCDD/Fs, dl-PCBs, PCNs, and CBz measured at these three sampling points are within the ranges of 0.71-44.9 ng/Nm³, 0.88-33.8 ng/Nm³, 0.78-41.8 ng/Nm³ and 127.4-6456.7 ng/Nm³, respectively. The results indicate that CBz formation are significantly higher than those of PCDD/Fs, dl-PCBs and PCNs. Besides, the removal efficiencies of all chlorinated aromatic compounds achieved with ACI+BH (95-99%) are significantly higher than those achieved with SCC+QT (71-89%) (Figure 2). Interestingly, the removal efficiencies of CBzs are significantly higher than those of PCDD/Fs, dl-PCBs and PCNs since the structure of CBzs are simple and easily destroyed in high operation temperature of SCC+QT. The total TEQ concentrations of PCDD/Fs, dl-PCBs, PCNs measured at three sampling locations are within the ranges of 0.05-4.62 ng I-TEQ/Nm³, 0.02-2.63 I-TEQ/Nm³ and 0.02-3.23 ng TEQ/Nm³, respectively.

**Conclusion:** Formation potential and removal characteristics of chlorinated aromatic compounds (PCDD/Fs, dl-PCBs, PCNs and CBz) from an SCS plant located in Taiwan are investigated. The results indicate that the concentrations of multi-pollutants emitted from this copper sludge smelting plant are significantly lower than those measured before improvement.

**Acknowledgments:** This study was financially supported by Taiwan MOST (Project no. MOST-105-2221-E-008-006-MY3)


**THU-PM2-C2 Release and Transformation of Chlorinated Paraffin during Thermal Treatment of Waste Plastics**

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**Introduction:** Chlorinated paraffins (CPs) are synthetic industrial products that are widely used as plasticizers and flame retardants for polymeric materials such as PVC and rubber. The thermal treatment process of technical CPs products has been studied (CP-52 and CP-70), and it has been concluded that CPs can decompose to generate chlorinated aromatic hydrocarbons such as PCBs and PCNs under heating conditions above 200 °C, and the generation pathway has been derived. However, there are still relatively few studies on the heat treatment of waste plastics in real life, and waste plastics not only generate UPOPs but also may release CPs under heating, and the released CPs may also act as precursors of UPOPs, making the generation of UPOPs increase. We mainly examined the release characteristics of SCCPs and MCCPs and the generation characteristics of UPOPs (such as PCBs, PCNs, and PCDD/Fs) during the heat treatment of waste plastics, and further constructed the link between the released CPs and generated UPOPs to derive the generation pathways of UPOPs after heat treatment of waste plastics.

**Materials and Methods:** Eight common plastic and rubber products, including PVC cables (with and without Cu cores), PE cling film, rubber tire skins and so on, were heat treated at 200-800 °C. The released SCCPs and MCCPs were detected by GC×GC-ECNI-QMS, and the generated PCBs, PCNs and PCDD/Fs were detected by HRGC-HRMS.

**Results:** We found that most of the materials reached the maximum CPs release before 400 °C. There is the maximum generation of PCBs and PCNs at 600-800 °C. As the release of CPs decreases, the generation of PCBs and PCNs increases, which can indicate, to some extent, that CPs are involved in the generation of UPOPs. The maximum generation of PCDD/Fs is at 300-400 °C, and the ratio of PCDFs/PCDDs is greater than 1, which can initially determine that the generation of PCDD/Fs after heat treatment of waste plastics is a de novo synthesis reaction. Furthermore, we conclude that PCBs and PCNs are mainly low-chlorine substituents, indicating that they are small molecule condensation processes. And PCDD/Fs are mainly high-chlorine substituents. In addition, by comparing the results of PVC cable skin and PVC cable (containing Cu core), we found that metallic copper can promote the decomposition and conversion of MCCPs, and can promote the mass production of PCDD/Fs and further chlorination of UPOPs.

**Discussion and Conclusion:** The release of CPs from SCCPs and MCCPs decreased gradually with increasing temperature at 200-400 °C, but the release of CPs from technical CPs products ceased after 400 °C, and the release of CPs from PVC products continued to increase again. This indicates that PVC is capable of generating CPs by thermal decomposition under high temperature conditions. In addition, the pathways of generating PCBs and PCNs after thermal treatment of CP-52 and waste plastics are consistent, both being generated by small molecule condensation. The more complex composition in the waste plastic led to the generation of PCDD/Fs after heat treatment, while the technical CPs products did not.

**Acknowledgments:** This research was funded by the National Natural Science Foundation of China (22276008)

**References:**
THU-PM2-C3  Comprehensive semiquantitative evaluation of Organochlorine compounds in Dioxin-contaminated soil samples from past Chlorine production processes by GC-TOFMS

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Introduction: One of the processes with the highest total historical release of PCDD/PCDF is the production of chlorine by chlor-alkali electrolysis using graphite electrodes (Rappe et al. 1990, Weber et al. 2008). As a comprehensive investigative approach for the presence of unintended organic residues at such sites, soil samples contaminated by the Japanese and German chlor-alkali processes, as well as another historical chlorine production process (the Weldon process associated with the Leblanc industry), were analyzed with high full scan sensitivity and GC-HR-TOF-MS, which provides accurate mass spectra at high spectral acquisition rates, and initial results have been published in conferences and papers (Takasuga et al. 2009, 2020).

In this study, chlorinated aromatic compounds previously identified by GC-HR-TOF-MS data were quantitatively evaluated with reference materials for the first time.

Materials and Methods: Information on the samples is detailed in an earlier paper. Soil samples with high PCDD/PCDF concentrations (50-150 ng TEQ/g) from a government survey and selected soil samples from a remediation project at the former Leblanc plant (Lampertheim/Germany) where chlorine production was conducted, were selected from the chlor-alkali site Rheinfelden/Germany site where high PCDF concentrations were detected (Otto et al. 2006;). Soil samples selected from a remediation project at the former Leblanc plant (Lampertheim/Germany), where chlorine was produced (Balzer et al. 2007). Using mass spectrometry software on GC-TOF-MS measurement data, mass chromatograms are generated from precise mass information of chlorinated aromatic compounds (CL-PAHs), including dioxins, and mass spectra are further analyzed to confirm that they are the substances for comprehensive analysis. Non-chlorinated polycyclic aromatic hydrocarbons (“PAHs”) were also targeted. Among the CL-PAHs detected in the mass chromatogram, CL-Benzene, CL-Benzonitrile, PCN, PCB, CL-Carbazole, and PCDF were quantified using PCN standards, while CL-Phe/Ant, CL-Flu/Pyr, CL-BaA/Chr, CL-BaP /BbF were quantified using CL-PAHs standards synthesized at the University of Shizuoka, and PAHs were quantified using PAHs standards.

Results and Discussion: A large number of chlorinated CL-PAHs were identified. Although the percentage of each CL-PAHs present and the degree of chlorination differed between samples, the composition of each detected CL-PAH isomer and isomers with the same number of chlorines was found to be nearly constant. It was suggested that these CL-PAHs may be contaminated by similar formation mechanisms. The degree of chlorination of CL-PAHs varied, but the low chlorinated component accounted for the majority. Identification accuracy was very high based on accurate mass spectra and mass chromatograms as well as existing analytical techniques. In particular, the exact mass measurements of the mass spectra obtained deviated only a few mDa or less from the theoretical values of the corresponding compounds.

The most frequently detected components were PCN, CL-(fluoranthene)/pyrene, CL-phenanthrene/(anthracene), CL-benz[a]anthracene/chrysene, CL-benzo[a]pyrene/benzo[b]fluoranthene and PCDF. On the other hand, concentrations of PCBs, chlorobenzene (CBz), and CL-carbazole tended to be lower than the other components listed above. Although many 2- to 6-ring PAHs components were identified, it is important to consider that PAHs such as naphthalene are easily vaporized and may also vaporize in the soil environment or during the sample storage and analysis processes, resulting in lower concentrations in the samples. A number of common PAHs were identified in each sample, but there were no clear differences among the samples.

The components identified were, in order of abundance, fluoranthene, pyrene, phenanthrene, and benzo[b]fluoranthene. In terms of the relationship between the concentrations of PAHs and CL-PAHs, the concentrations of CL-PAHs tended to be higher than those of PAHs, suggesting that chlorination of the backbone PAHs was in progress. The chromatograms of CL-PAHs for each sample showed relatively similar isomer distributions.

THU-PM2-C4  Catalytic Pyrolysis of Dioxins in MSWI Fly Ash: Mechanism and Application

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1. Introduction:
The amount of municipal solid waste (MSW) has increased to 249 million tons in 2021 and incineration has become the mainstream disposal method in China. According to the data of National Bureau of Statistics, China, the incineration ratio has rapidly increased from 43.8% in 2017 to 72.5% in 2021. However, 3-5% of fly ash (FA) was produced in the incineration process and as a result about 10 million tons of FA were generated in China. Recycling of fly ash for further application was very limited due to the high concentrations of heavy metals, chlorides and dioxins. Various technologies have been investigated to reduce the potential toxic elements (PTEs), such as sintering, vitrification and cement kiln co-processing. The cement kiln co-processing can fix or destroy the PTEs at 1300 ~1450°C in an alkaline atmosphere. However, cement kiln co-processing requires a shadow chlorine content of fly ash (< 0.04 wt.%), which restricted the utilization of fly ash in cement production². Sintering and vitrification of fly ash was adopted to produce ceramsite and vitreous bodies, however, the secondary pollution problems and high cost restricted their application³. Therefore, utilization of fly ash is still limited and more than 90% of fly ash ends up in sanitary landfilling. Especially, dioxins, has attracted increasing attention due to the highly hazardous to the environment and human health. In addition, the limit of dioxins in fly ash that is considered as safe to use is 50 ng-TEQ/kg according to the Chinese technical specification for pollution control of fly-ash from municipal solid waste incineration⁴. Hagenmaier et al. conducted the first research on fly ash treatment using pyrolysis system and significant reduction was achieved⁵. Trinh and Chang studied the effect of Pd catalyst on dioxins destruction, which can improve the pyrolysis process and remove the dioxins in a shorter residence time⁶. However, the noble metal catalyst is still too expensive for industrial application. In this study, we investigated the effect of water washing on dioxin decomposition in fly ash and the distribution of dioxins in flue gas in the pyrolysis process. In addition, the influence of Fe/C, V2O5-WO3/TiO2 and CaO additives was compared by analyzing the residual dioxins and fingerprint signature. Moreover, the catalytic pyrolysis of dioxins in municipal solid waste incineration fly ash has been verified and applied to industrial production.

2. Materials and Methods:
Fly ash. The raw FA samples were collected from baghouse filter of an incinerator in Zhejiang province, China. Prior to the experiments or analyses, all fly ashes were dried in an oven at 105°C for over 24 h. An exact 50.00 g raw FA was washed with DI water (ratio of 10 mL:1 g) at room temperature with magnetic stirring (200 rpm) for 30 min. Solid-liquid separation was carried out by vacuum suction filtration, and the solid obtained after suction filtration was dried to obtain the water washed FA (designated as washed fly ash, WFA). The main composition of FA and WFA were analyzed via X-ray fluorescence (XRF, Thermoscientific ARL ADVANT’X IntelliPowerTM 4200, USA), and the soluble chlorine in fly ash was determined by ion chromatography, and the results are shown in Table 1.

<table>
<thead>
<tr>
<th></th>
<th>Ca</th>
<th>Cl</th>
<th>Na</th>
<th>K</th>
<th>Si</th>
<th>Mg</th>
<th>Al</th>
<th>Fe</th>
<th>Zn</th>
<th>soluble chlorine</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA</td>
<td>25.94</td>
<td>21.14</td>
<td>9.71</td>
<td>4.29</td>
<td>4.05</td>
<td>1.34</td>
<td>1.25</td>
<td>1.51</td>
<td>0.73</td>
<td>12.07</td>
</tr>
<tr>
<td>WFA</td>
<td>34.14</td>
<td>1.21</td>
<td>0.91</td>
<td>0.61</td>
<td>9.07</td>
<td>3.43</td>
<td>2.97</td>
<td>3.43</td>
<td>1.36</td>
<td>0.62</td>
</tr>
</tbody>
</table>

Catalytic pyrolysis test. A laboratory-scale pyrolysis module was designed and constructed as described in Fig. 1. With quartz boat used as the container, exact 4.00 g FA or WFA was placed in a quartz tubular reactor for pyrolysis. Carrier gas was controlled by a mass flow controller. Before starting the experiment of each batch, the reactor was purged with N2 at a flow rate of 1,000 sccm for 30 min to completely eliminate the residual O2 from the system. The experiments were conducted at 200°C, 250°C, 300°C, 350°C, respectively, for 1 h under a 200 ml/min flow of N2 to evaluate the temperature effect. The PCDD/Fs in off-gas were adsorbed by XAD-II polymeric resin combined with toluene. In addition, 10 wt.% of Fe/C, V2O5-WO3/TiO2, and CaO was added into the fly ash to improve the pyrolysis, respectively. After experiment, the quartz boat is taken out of the tube to cooled down in a N2 chamber. To minimize the influence of the reactor, the quartz tube and reactor were rinsed with acetone, toluene, hexane and dichloromethane before each experimental test, and then washed with ultrasonic bath after each experimental test.
Large-scale application. A 40 ton/day catalytic pyrolysis equipment was constructed and is currently operational in Huzhou, Zhejiang. The equipment is divided into two levels of spiral, with the upper level mainly used to dry the fly ash, and the lower level used to thermally decompose the dioxins in the fly ash. The operating temperature of the equipment is 350°C, and the oxygen concentration is controlled below 0.5%. The thermal treated fly ash is indirectly cooled by water to 150°C to avoid the re-synthesis of dioxins.

3. Results:
Effect of water washing on dioxin decomposition. The dioxin concentration in the raw fly ash was 427.24 ng I-TEQ/kg. Soluble chloride, such as NaCl, KCl, was removed during the washing process, resulting in a mass loss of 38.82%. As shown in Table 1, the soluble chlorine reduced from 12.07% to 0.62%. The dioxins concentration in the WFA increased to 695.44 ng I-TEQ/kg, which increased by 62.77%, in line with the proportion of mass loss in the fly ash. Dioxins were difficult to be dissolved in water, and the total amount of dioxin in the washing liquid accounts for only 0.233% of the raw fly ash.

The effect of water washing on dioxins decomposition in fly ash was investigated at 200°C, 250°C, 300°C, and 350°C, which was shown in Figure 2. Although water washing pretreatment enriches and increases the dioxins concentration in the fly ash, the TEQ decomposition efficiencies for the WFA were higher than the raw FA at same temperature. The decomposition rates of dioxins at 200°C, 250°C, 300°C, and 350°C were 56.1%, 99.3%, 99.5%, and 99.6%, respectively, indicating a higher thermal decomposition efficiency. When the WFA was thermally treated at 250°C for 10 minutes, the concentration of dioxin decreases to 4.85 ng I-TEQ/kg, which meets the EU standard (20 ng I-TEQ/kg). Water washing pretreatment can not only reduce the reaction temperature, but also significantly improve the thermal decomposition efficiency, thus reducing energy consumption. In addition, the dioxins in the flue gas was also analyzed, and the concentration was 0.4 ng TEQ/m3, which accounts for only 0.09% of the raw fly ash.

Figure 2: Degradation efficiency of dioxins for raw and water-washed fly ash.
Effect of additives on dioxin decomposition. To lower the temperature for dioxins decomposition, Fe/C, VWTi and CaO additives was added in the fly ash. Thermal decomposition efficiency of dioxins for washed fly ash was 70.5%, 88.9% and 94.8% respectively. The additives significantly enhanced the degradation efficiency of dioxins in low-temperature thermal decomposition. It can be seen that CaO additive has the best low-temperature thermal decomposition effect, and the concentration of fly ash decreased to 25.67 ng-TEQ/kg at 210°C. The use of CaO additive can further reduce the reaction temperature of low-temperature thermal decomposition. Comparison was made among CaO additive addition ratios of 1 wt.%, 5 wt.%, and 10 wt.%. As shown in Figure 3, after treated at 210°C for 10 minutes, the dioxin concentrations in the WFA were 165.55 ng-TEQ/kg, 110.93 ng-TEQ/kg, and 25.67 ng-TEQ/kg, and the dioxin degradation efficiencies were 76.19%, 84.05%, and 96.31%, respectively. It can be seen that as the addition ratio increases, the dioxin concentration in the fly ash decreases.

Decomposition of OCDD. Simulated fly ash with pure OCDD was used to study the decomposition mechanism of the dioxins. Firstly, the fly ash was extracted with toluene and cleaned with acid/alkaline aluminum oxide column to remove the dioxins and precursors. 10 ng of OCDD was added to 1g fly ash, as a result the OCDD concentration was measured as 9323.14 pg/g. At 200°C, OCDD decreased by 2228.65 pg/g, with a degradation rate of 23.9%. At the same time, the low-chlorinated PCDDs congeners increased significantly. Among them, 1,2,3,4,6,7,8-HpCDD was generated at 1654.95 pg/g. This suggests that the C-Cl bond at the substitution position closest to the oxygen atom of OCDD is the most unstable and undergoes dechlorination firstly. From Table 2, At 200°C only the dechlorination occurs, and the high-chlorinated dioxin homologues were converted into low-chlorinated dioxins. When the reaction temperature rose to 250°C, decomposition reaction of dioxin dominates.

Table 2: Distribution of dioxin congeners before and after thermal decomposition

<table>
<thead>
<tr>
<th>Compound</th>
<th>Isomer</th>
<th>initial</th>
<th>temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>200°C</td>
</tr>
<tr>
<td>T4CDD</td>
<td>2378</td>
<td>2.64</td>
<td>13.47</td>
</tr>
<tr>
<td>PcCDD</td>
<td>12378</td>
<td>9.93</td>
<td>79.10</td>
</tr>
<tr>
<td>H6CDD</td>
<td>123478</td>
<td>5.39</td>
<td>58.74</td>
</tr>
<tr>
<td>H6CDD</td>
<td>123678</td>
<td>14.91</td>
<td>131.59</td>
</tr>
<tr>
<td>H6CDD</td>
<td>123780</td>
<td>10.45</td>
<td>170.98</td>
</tr>
<tr>
<td>H7CDD</td>
<td>1234678</td>
<td>148.69</td>
<td>1883.64</td>
</tr>
<tr>
<td>O8CDD</td>
<td>12346789</td>
<td>9323.14</td>
<td>7094.49</td>
</tr>
<tr>
<td>PCDDs</td>
<td>9515.15</td>
<td>9352.01</td>
<td>141.55</td>
</tr>
<tr>
<td>PCDFs</td>
<td>378.46</td>
<td>460.98</td>
<td>51.32</td>
</tr>
<tr>
<td>TOTAL</td>
<td>9895.60</td>
<td>9812.99</td>
<td>192.87</td>
</tr>
</tbody>
</table>

As shown in Figure 4, with the assistant of CaO, the dechlorination rate of hexa-, hepta-, and octa-chlorinated PCDD was accelerated at 200°C. CaO additives reacted with the removed Cl to promote the dechlorination reaction of highly chlorinated dioxins.

\[ CaO + 2HCl \rightarrow CaCl_2 + H_2O \]
Application. In the 40 ton/day catalytic pyrolysis equipment, catalytic pyrolysis of dioxins in fly ash was investigated. The initial concentration varies from 150 to 1340 ng TEQ/kg. At 350°C dioxins can be decomposed to be lower than 50 ng TEQ/kg, which meet the standard. However, it is difficult to pyrolysis the dioxins lower than 10 ng TEQ/kg, which can be easily achieved in laboratory. One possible reason for this could be that it was difficult to maintain a zero level of oxygen concentration in the large equipment. The presence of water in fly ash can contribute to the formation of dioxins through de novo synthesis. This occurs when the residual oxygen in the fly ash reacts with organic compounds in the presence of heat and water vapor.

4. Discussion:
Reducing chloride content in fly ash using water washing as a pretreatment method results in enhancement of PCDD/Fs decomposed in fly ash using pyrolysis is confirmed in this work. And the distribution of dioxins in the residue fly ash and washing liquid was analyzed. In addition, the dioxins in the flue gas in the pyrolysis process accounts lower than 1% of the raw fly ash, which can be easily removed by the air pollution control devices. In the previous study, noble metal catalysts were used to improve the pyrolysis efficiency, which is quite expensive for industrial application. However, this work proved that free CaO can enhance the dioxins decomposition by reacting with the chlorine. The pyrolysis of pure OCDD indicated that high-chlorinated dioxin congeners were dechlorinated to lower chlorinated dioxins at 200°C. And at higher temperature, destruction reaction of dioxin, such as the break of the C-O bond dominates. Moreover, we have successfully applied catalytic pyrolysis to industrial production and have been able to decompose dioxins in fly ash on a large scale.

5. Conclusions:
Even though the catalytic pyrolysis has been applicated in industry, the system needs to be optimized to enhance the pollutants destruction efficiencies and lower the cost. In addition, advanced heavy metals stabilization coupled with pyrolysis process in a low-carbon way should be developed to meet the requirement for resource utilization of treated fly ash.

6. Acknowledgments:
This work was supported by the Key R&D Program of Zhejiang Province, China (No. 2022C03056).

7. References:
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Wageningen Food Safety Research, Wageningen University & Research, Akkermaalsbos 2, 6708 WB Wageningen, The Netherlands

**Introduction:** Per- and polyfluoroalkyl substances (PFASs) are used in various industrial and commercial applications, including food contact materials (FCMs). Sensitive and reliable methods for PFAS detection in FCMs are important due to their persistence and potential health risks. Besides fluorinated carboxylates and sulfonates, especially for FCMs, also other PFASs are relevant. This group includes fluorotelomer alcohols (FTOHs) and polyfluoroalkyl phosphate acid monoesters (mono-PAPs). The analysis of FTOHs is challenging due to their volatility, tendency to aggregate in the presence of salts, and difficulty in ionization [1]. FTOHs are generally analyzed using GC-MS/MS [2] or after derivatization on LC-MS/MS [3], requiring additional extraction and/or derivatization steps. Mono-PAPs, a group of PFASs with divalent charges on a phosphate group, are very difficult to separate chromatographically [4]. Here we report on a method that uses a single extraction step without clean-up, followed by two separate LC-MS/MS methods for the detection of 47 PFASs, including acids, sulfonates, FTOHs, mono- and di-PAPs, sulfonamides, and telomer sulfonates. The developed methods were applied to the analysis of 78 FCMs, including paper, cardboard, plant material, and plastic.

**Materials and Methods:** 0.5 g of the FCM was weighed, and internal standards were added. The extraction was carried out using methanol. After shaking, ultrasonication, and centrifugation, a small portion of the extract was combined with water and injection standard. The samples were then filtered prior to measurement. The FTOHs were separated on a mixed-mode C18 column with positive surface modification and methanol and water as the mobile phase. They were analyzed on a tandem mass spectrometer. The other PFASs were separated on an end-capped C18 column, utilizing an alkaline aqueous mobile phase (0.1% NH₄OH + 2 mM NH₄OAc buffer) and acetonitrile.

**Results:** The alkaline mobile phase enhanced the peak shape of the mono-PAPs by driving them to their fully deprotonated form. Additionally, the alkaline conditions improved ionization in negative ESI mode, making the method more sensitive compared to neutral buffered methods. The method included 47 PFASs, comprising also FTOHs and mono-PAPs, of which 41 complied with our quality criteria for quantitative analysis in the tested materials. The methods demonstrated good selectivity, linearity, and accuracy. The limit of quantification for 6:2-, 8:2-, and 10:2-FTOH were 6.25, 25, and 50 ng/g, respectively, while the limit of quantification for other PFASs ranged from 25-250 pg/g. PFAS concentrations in the tested FCMs ranged up to ~1600 ng/g (6:2-FTOH). The highest concentration of PFOS was 32 ng/g, which exceeds the limit of 25 ng/g as stated in Commission Delegated Regulation (EU) 2020/784. Apparent recoveries ranged from 81-129% (average: 99%) for the quantifiable analytes.

**Discussion and Conclusion:** The developed method for the detection of PFASs in FCMs offer several advantages over previously published methods. One significant advantage is the use of a single extraction step without the need for any clean-up, which is efficient and cost-effective. Additionally, the method has the capability to detect a broad spectrum of PFASs, including FTOHs and mono-PAPs, without the need for derivatization. The developed methods can reliably quantify 41 PFASs, including FTOHs and mono-PAPs, at levels well below the limits stated in Commission Delegated Regulation (EU) 2020/784. In our current study, we found similar levels of 6:2-FTOH as published by Yuan et al. (2016) and Liu et al. (2015) in our tested FCM. The tested FCM in this study did not contain significant concentrations of 8:2- and 10:2-FTOH, however.

To further advance this work, future studies should focus on the analysis of a wider range of FCMs to provide a better understanding of PFAS presence in packaging materials. Researchers should also explore new ways to detect more species of PFASs in a single analysis. Overall, the methods developed in this research have the potential to provide an effective tool for monitoring and regulating PFASs in FCMs, thereby improving public health and environmental protection.

**Acknowledgments:** The Dutch Food and Consumer Product Safety Authority (NVWA) and the Ministry of Agriculture, Nature and Food Quality are gratefully acknowledged for providing the samples, and for funding this research (WOT-02-006-016).

**References:**
AFTERNOON BREAKOUT SESSIONS II

THURSDAY 14 SEPTEMBER 2023

15:40-17:00

Food Contact Materials

J.Van Loco & H.Vanderperren

THU-PM2-D2  Assessment of poly- and perfluoroalkyl substances (PFAS) in commercially available drinking straws using targeted and suspect screening approaches

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4 Behavioural Ecology and Ecophysiology Group, University of Antwerp, Universiteitsplein 1, 2610, Wilrijk, Belgium

Introduction: Intake through food and drinking water are the main routes of general human exposure to poly- and perfluoroalkyl substances (PFAS). Many food packaging materials (FCMs) and reusable plastic bags, used in food industry, can contain PFAS [1]. A recent study in the U.S.A. [2] revealed that PFAS can also be found in straws made from plant-based materials. Considering that a study on PFAS in plant-based straws has never been explored in Europe, we investigated PFAS concentrations in commercially available straws from the Belgian market and extended the American study on paper straws [2] with straws made from bamboo, glass stainless steel and plastic. We subjected 39 different brands of straws to an extensive targeted analysis for 29 individual PFAS. In addition, we performed suspect screening on a subset of straws to investigate the presence of other non-targeted or new generation PFAS.

Materials and Methods: Based on the available market supply, we subjected 20 brands of paper straws, five brands of glass straws, five brands of bamboo straws, five brands of stainless steel straws, and four brands of plastic straws to targeted analysis of 29 PFAS using UPLC-MS/MS. Three replicates were included per brand. A solvent (methanol) extraction method, based on Powley et al. [3], followed by sample clean-up using granular activated carbon powder, was used to extract PFAS from the straws. A selection of straws, selected based on the PFAS concentrations and diversity in PFAS profiles, were subjected to suspect screening using LC-QTOF-MS. These included one glass, one plastic, three bamboo, and eight paper straw brands. Two suspect lists (>10000 PFAS) were used to identify the suspect features.

Results: Targeted analysis revealed that 16 out of the 29 PFAS were quantified in the different types and brands of straws. PFAS were detected in almost all brands of paper (LOQ – 7.15 ng/g) and bamboo (<LOQ – 3.47 ng/g) straws. Furthermore, PFAS were detected in two brands of glass straws (<LOQ – 6.65 ng/g) and three brands of plastic straws (<LOQ – 0.924 ng/g). Stainless-steel straws did not contain quantifiable levels of any of the targeted PFAS. There was a significant variation in PFAS profiles between straws from the same materials. Two compounds were newly identified as confidence level 1 (i.e. confirmed using reference standard) by suspect screening (i.e. trifluoroacetic acid (TFAA) and trifluoromethanesulfonic acid (TFMS), which are both ultra-short chain PFAS. Both compounds were only detected in plant-based straws.

Discussion and Conclusion: The presence of PFAS in plant-based straws was expected as PFAS are known to be used to confer stain and water repellency to FCMs [4]. Stainless steel is often made of metal-oxides and thus has no net charge on the surface of the straws, resulting in limited or no adsorption to this material. Some glass straws are made of borosilicate glass so the presence of PFAS in these straws might be due to adsorption to silica minerals [5]. Our study confirmed that particularly plant-based straws might be an exposure route of PFAS, including ultra-short chain PFAS, which might result in increased exposure to PFAS for humans.

Acknowledgments: This work was partly supported by the Research Foundation Flanders (FWO, grants G018119N) and partly by the Exposome Centre of Excellence of the University of Antwerp (BOF grant, Antigoon database number 41222). TG is junior post-doctoral researcher of the FWO (grant nr. 12ZZQ21N).

References:
Food Contact Materials
J.Van Loco & H.Vanderperren

THU-PM2-D3 Investigation of potential migratables from paper and board food contact materials intended for takeaway

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1. Introduction:
Since the ban on single-use plastic articles in Europe, the food contact material (FCM) industry has been forced to move to more sustainable alternatives. All plastic packaging should be reusable or easily recyclable by 2030 in accordance with the EU strategy for Plastics in the Circular Economy. Various alternatives have been developed, focusing on bioplastics or recycled materials. Alternatively, the applications of other materials have been extended. For example, straws made of single-use plastic have been replaced by paper straws. Paper and board FCM offer a significant advantage, i.e., versatility. Indeed, depending on the coating or treatment of the paper and board material, they can be used for liquids, frozen, fresh, or dry foods. In addition, paper and board is the main material used in fast-food restaurants. Although paper and board FCM are convenient alternatives, they must be safe for consumers. Therefore, it is crucial to evaluate the migration of potentially harmful substances. These substances can be intentionally added, e.g., additives, synthetic fibers, adsorbents, treatment agents, and colorants, or be present unintentionally, like degradation products or substances originating from the recycling process. Furthermore, paper and board FCMs are often coated, glued, printed, composed of several layers, or combined with other materials, so the final FCM contains even more potential. Over the past ten years, paper and board FCM have been studied extensively. Many substances, such as phthalates (1-3), printing inks (4,5), bisphenols (6–8) as well as mineral oil (9-11), were often reported in the literature as contaminants from paper and board FCM. However, limited data exist on takeaway articles or straws, although they are gaining popularity, especially since the Covid-19 crisis. Therefore, assessing the risk of these FCM, frequently used in daily life, is crucial. Accordingly, this study aims to investigate and assess the risk of potential migrations of bisphenols, plasticizers, primary aromatic amines, photoinitiators, and mineral oil from straws and takeaway articles made of paper and board.

2. Materials and Methods:
First, 20 straws and 58 takeaway articles were purchased on the Belgian market in supermarkets, dedicated shops for consumers and professionals and fast food restaurants. Then, extraction experiments based on the CEN standards EN 645 (12) & EN 15519 (13) were applied to the samples. The intact article was extracted when article filling (e.g., cups, bowls) or immersion (e.g., straws) was possible. If not possible, one dm² was cut and immersed in the extraction solvent (water or 95% ethanol). In total, 25 primary aromatic amines, 20 photoinitiators and five bisphenols were analysed in the water extract (EN 645) by LC-MS/MS, while the analysis of 14 plasticizers was performed in the ethanol extract (EN 15519) by GC-MS/MS. For the analysis of the saturated and aromatic fraction of mineral oil (MOSH and MOAH, respectively) by GC-FID, the BFR procedure (14) was applied. Next, the extraction results were used to determine the exposure of the consumers, thereby applying different hypotheses on the consumption of food intended to be in contact with the targeted articles. Finally, the risk assessment was performed according to the RACE tool (Rapid Assessment of Contaminant Exposure tool), developed by EFSA. First, toxicological information (e.g., Health Based Guidance Values) of the migrants was collected from different sources. If a Health Based Guidance Value was not available, a reference point (e.g. No Observed Adverse Effect Level (NOAEL)) was searched. In cases where no, or very limited, toxicological information is available, the threshold of toxicological concern (TTC) approach was used. Thereafter, according to the EFSA guidance, this study targeted three categories of age: Children (3-10 years old, 23 kg), teenagers (14-18 years old, 61 kg) and adults (18-64 years old, 70 kg). In order to perform a proper risk assessment, hypothesis on consumption of food intended to be in contact with the targeted articles were determined.

3. Results:
One of the categories with the largest shift from single-use plastics towards paper and board is the category of takeaway articles and straws. Therefore, a representative sampling of these types of FCMs was performed. Overall, 20 straws for cold and hot use were selected. Regarding takeaway FCM, 58 samples were purchased, covering boxes (e.g. hamburger, pizza, noodle), trays/bags (e.g. fries, snack), wraps/bags (e.g. hamburger, sandwiches, tacos), cups (hot and cold use), bowls (e.g. soup, salad, ice cream) and utensils (e.g. spoon).
First, the migration of primary aromatic amines was investigated. Out of the 25 targeted primary aromatic amines, only 3,3’-dimethylbenzidine (3,3’-DMB) was found in three takeaway samples (i.e. one pizza box, one noodle box, and one hamburger box) with concentrations ranging from 0.00032 mg kg\(^{-1}\) to 0.00052 mg kg\(^{-1}\).

Next, the migration of bisphenols was also investigated. Five different substances were targeted in this study (BPA, BPS, BPF, BPZ, and BPB). Only BPA and BPS were detected in 11 takeaway samples with concentrations ranging from 0.005 mg kg\(^{-1}\) up to 0.026 mg kg\(^{-1}\) of BPA and from 0.008 mg kg\(^{-1}\) up to 0.017 mg kg\(^{-1}\) of BPS. No bisphenols were present in straw sample.

The presence of photoinitiators could also be expected. Out of the 20 photoinitiators targeted, only benzophenone (BP) and 1-hydroxycyclohexyl phenylketone (HCPK) were found in 7 takeaway articles, and HCPK was present in one straw. BP levels ranged from 0.002 mg kg\(^{-1}\) up to 0.006 mg kg\(^{-1}\) and from 0.003 mg kg\(^{-1}\) up to 0.021 mg kg\(^{-1}\) of HCPK.

Next, the migration of plasticizers was investigated. Three plasticizers (DBP, DiBP, and DEHP) were present in straws and takeaway articles. Additionally, four more plasticizers (BBP, DiDP, DINP, and DINCH) were detected in the takeaway articles. Overall, 60% of the straws contained at least one phthalate compared to 56% for takeaway articles. However, more plasticizers were found in takeaway articles. An overview of the results is given in Table 1.

Finally, MOSH and MOAH (fractions C10 to C50) were investigated in 2 straws and 53 takeaway articles. MOSH and MOAH were found in the two straws with an average concentration of MOSH of 26.3 mg kg\(^{-1}\) and 0.32 mg kg\(^{-1}\) of MOAH. All the analysed takeaway articles contained MOSH, while MOAH was found in 88.7% of takeaway articles. More details on the results are given in Table 1.

### Table 1: Concentration of plasticizers, MOSH and MOAH found in takeaway articles and straws expressed in mg kg\(^{-1}\), minimum, maximum and median concentrations, count of samples with detection above the LOQ (n), percentage of samples in which the compound was quantified and the sample type with the highest concentration found.

<table>
<thead>
<tr>
<th>Substances</th>
<th>n</th>
<th>% of samples containing the substance</th>
<th>Minimum (mg/kg)</th>
<th>Maximum (mg/kg)</th>
<th>Median (mg/kg)</th>
<th>Sample type with highest concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Takeaway articles</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DiBP</td>
<td>28</td>
<td>36</td>
<td>0.006</td>
<td>0.46</td>
<td>0.017</td>
<td>Takeaway Box</td>
</tr>
<tr>
<td>DBP</td>
<td>19</td>
<td>24</td>
<td>0.005</td>
<td>0.07</td>
<td>0.025</td>
<td>Noodle box</td>
</tr>
<tr>
<td>DEHP</td>
<td>21</td>
<td>27</td>
<td>0.005</td>
<td>0.15</td>
<td>0.039</td>
<td>Pizza box</td>
</tr>
<tr>
<td>DiDP</td>
<td>1</td>
<td>1</td>
<td>0.01</td>
<td></td>
<td></td>
<td>Coffee cup</td>
</tr>
<tr>
<td>DINP</td>
<td>19</td>
<td>24</td>
<td>0.012</td>
<td>0.12</td>
<td>0.035</td>
<td>Noodle box</td>
</tr>
<tr>
<td>DINCH</td>
<td>14</td>
<td>18</td>
<td>0.006</td>
<td>0.011</td>
<td>0.023</td>
<td>Hamburger box</td>
</tr>
<tr>
<td>BBP</td>
<td>10</td>
<td>13</td>
<td>0.005</td>
<td>0.013</td>
<td>0.006</td>
<td>Fries cone</td>
</tr>
<tr>
<td>MOSH</td>
<td>53</td>
<td>100</td>
<td>0.01</td>
<td>35.9</td>
<td>0.83</td>
<td>Coffee cup</td>
</tr>
<tr>
<td>MOAH</td>
<td>47</td>
<td>88.7</td>
<td>0.01</td>
<td>1.76</td>
<td>0.16</td>
<td>Noodle box</td>
</tr>
<tr>
<td><strong>Straws</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DiBP</td>
<td>10</td>
<td>13</td>
<td>0.008</td>
<td>0.029</td>
<td>0.013</td>
<td>White and red straw</td>
</tr>
<tr>
<td>DBP</td>
<td>5</td>
<td>7</td>
<td>0.007</td>
<td>0.032</td>
<td>0.015</td>
<td>White and red straw</td>
</tr>
<tr>
<td>DEHP</td>
<td>9</td>
<td>12</td>
<td>0.011</td>
<td>0.049</td>
<td>0.041</td>
<td>Bicolor straw</td>
</tr>
<tr>
<td>MOSH</td>
<td>2</td>
<td>100</td>
<td>1.5</td>
<td>51.0</td>
<td>26.2</td>
<td>Black Paper straw for cold beverages</td>
</tr>
<tr>
<td>MOAH</td>
<td>2</td>
<td>100</td>
<td>0.05</td>
<td>0.59</td>
<td>0.3</td>
<td></td>
</tr>
</tbody>
</table>
Although new FCMs can be innovative and environmentally friendly, they should also be safe for consumers. However, contaminants of concern can still be present, potentially migrating into food. Since no specific EU legislation exists for paper and board, a risk assessment was performed on all migrants. Based on the migration results and considering the exposure scenarios, the potential risks were assessed for children, teenagers and adults. Overall, the results brought to light potential concerns for consumers for bisphenol A, 3,3′-DMB, DIBP and mineral oil hydrocarbons and this for several sample categories. Nevertheless, children are more at risk than teenagers or adults for almost all the substances in the different categories of samples. However, MOAH is the substance that can potentially be of concern for consumers in many article categories, regardless of their age and the scenario considered. Regarding MOAH, two scenarios were considered in accordance with the latest draft scientific opinion on the update of the risk assessment of mineral oil hydrocarbons in food by EFSA (15), that is currently under public consultation. These two scenarios consider the BMDL10 of 0.49 mg kg\(^{-1}\) bw per day, with average contents of 10% or 1% of 3- or more ring MOAH within the MOAH fraction, since this sub-fraction is considered the most toxic (15).

4. Discussion:
Although the migration of primary aromatic amines has been well studied in plastic FCM, this research is the first to investigate these substances in straws and takeaway articles made from paper and board. The presence of this substance can originate from different sources. It can be used as an intermediate for producing azo dyes and insoluble pigments in the paper industry or plastic coatings. The sample in which 3,3′-DMB was found (noodle and hamburger boxes) were coated with polylactic acid, while another sample (pizza box) was coloured, which could explain the presence of this primary aromatic amine. Overall, data gaps exist regarding the potential presence of bisphenols in straws and takeaway articles. The presence of BPA and BPS in paper and board can originate from different sources like recycling, coatings, or dyeing of the samples. Indeed, BPA can also be used as a color developer in thermal paper. Suciu et al. investigated the presence of BPA in boxes for frozen and takeaway pizza (9). BPA was found in all the pizza boxes. Compared with the results of the current study, higher concentrations of BPA were found in the study of Suciu et al. However, different conditions were used. Suciu et al. performed migration tests using the simulant Tenax\(^{®}\), while the experiments in the current study were performed according to EN 645 (i.e. extraction with water), which could explain the difference in the results.

Regarding photoinitiators, they are used in the UV curing processes of inks and lacquers applied to the packaging surface, mainly cardboard boxes and multilayers. Due to their volatility, these substances can migrate from the packaging and contaminate food. The majority of samples in which photoinitiators were found were coloured. For the uncoloured samples, the presence could originate from the recycled fibers. However, no information was available. Photoinitiators have been extensively analyzed in dry food. However, studies performed on paper and board FCM intended for takeaway are scarce and did not follow the same protocol. Therefore, the comparison of the results was not relevant.

Regarding plasticizers, in the FCM industry, these substances can be used to increase the flexibility of the materials and can also be part of printing inks and lacquers. When comparing the results to other studies, it was observed that plasticizers are frequently found in this type of material. A study conducted in 2007 already showed the presence of DiBP in 16 takeaway pizza boxes (2). The same year, another study showed the presence of DBP and DEHP in takeaway items (pizza, fries bags etc.) (3). More recently, in 2013, Suciu et al. found DEHP in 50% of the pizza boxes analyzed in their study (9). However, the methodologies applied in these studies were again not comparable to the current study and therefore the observed concentrations cannot be compared.

Finally, the content of MOSH and MOAH in paper and board has already been well investigated for dry food sold on the market (11,16). However, limited data exist on takeaway articles or straws even if these substances can potentially impact human health since MOAH can act as genotoxic carcinogens, while MOSH can accumulate in human tissues. Fengler et al. studied the content of MOSH and MOAH in fast food packaging (10) and Conchione et al. in pizza boxes (11). These two studies applied a similar method of analysis as the present study. In the study of Fengler et al., The results of pizza boxes were comparable between both studies. In hamburger boxes, fries trays, and a wrap, the difference in concentration could be explained by a higher variability of samples found on the market. As only a limited number of samples have been analysed in the study of Fengler et al., a comparison of results with the present study would be biased. In the study of Conchione et al., samples were divided in two categories. Category I groups pizza boxes potentially made of virgin paper board while group II groups samples suspected to contain recycled fibers. In the present study, the results obtained for pizza boxes are more similar to the results of group II, potentially indicating that recycled fibers were present in the samples.

Finally, regarding the risk assessment, it should be noted that the obtained results can be considered a worst-case since they...
were based on extraction rather than migration, potentially overestimating the actual migration into food. Therefore it would be relevant to analyze the food itself (e.g., a pizza instead of a pizza box) to conduct a more realistic risk assessment.

5. Conclusions:
Seventy-eight food contact materials made of paper and board were analyzed to identify potential migrations of harmful substances to human health, such as plasticizers, bisphenols, photoinitiators, mineral oil, and primary aromatic amines. The extraction experiments highlighted the presence of 14 substances out of the 66 targeted in the samples. Photoinitiators were detected in 9% of the samples, bisphenols in 13%, primary aromatic amines in 5%, plasticizers in 56%, and mineral oil in 100%. A risk assessment was carried out for each migrant highlighting a potential concerns for the consumer for 5 substances in many FCM categories. This study demonstrates the importance of further and more realistic evaluation of these materials, in particular by migration tests or analysis of the food itself.

6. Acknowledgments:
This study was funded by the Belgian Federal Public Service Health, Food Chain Safety and Environment (MIGRACARTO).

7. References:
12. CEN. EN 645 : 1994 - Paper and board intended to come into contact with food stuffs - Preparation of a cold water extraxt. BSI Standards.
13. CSN. EN 15519 - Paper and board intended to come into contact with foodstuffs - Preparation of an organic solvent extract. Bundesinstitut für Risikobewertung. 2012, Determination of hydrocarbons from mineral oil (MOSH & MOAH) or plastics (POSH & PAO) in packaging materials and dry foodstuffs by solid phase extraction and GC-FID.
Identification of plastic additives and non-intentionally added substances in food packaging materials by thermal desorption coupled to GC-MS

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1. Introduction:
World production of plastics continues to increase steadily, with a total output of 390.7 million tonnes in 2021. Approximately 44% of this production is assigned to packaging applications, with a significant proportion being allocated for food and beverages (Plastics Europe, 2022). Plastic materials employed in this application are diverse, encompassing a range of polymers such as polyethylene (PE) and polyethylene terephthalate (PET), polypropylene (PP), polyvinyl chloride (PVC), or polystyrene (PS) (Guart et al., 2011). In some cases, a combination of these polymers is utilized, such as poly(ethylene:propylene) copolymers. To enhance the mechanical and physicochemical properties of plastics, the incorporation of additives is necessary. The primary additives utilized, based on their respective global market shares, include plasticizers, fillers, flame retardants, antioxidants, thermal stabilizers, impact modifiers, colorants, lubricants, and UV stabilizers (Cheng et al., 2020). Orthophthalates were usually the most common plasticizers used in food packaging. However, some compounds of this group are restricted in some applications due to their reproductive toxicity and endocrine disrupting effects on human health (Carlos et al., 2018), for instance, children’s products, toys, cosmetics, or medical devices. Citric acid esters (CAEs) have become one of the main group of compounds used as alternative plasticizers to phthalates (Zhang et al., 2022), as well as adipates or 1,2-cyclohexanediacarbonylic acid, dinonyl ester DINCH (Edwards et al., 2021). Acetyl tributyl citrate (ATBC) is currently the main substitute due to their improvement in mechanical properties of materials such as PVC (Liu et al., 2023), and is also used as a pharmaceutical excipient (Kim et al., 2018). The lack of toxicity, negative test in carcinogenic effects and better properties as a plastic additive have led to a major use in a wide variety of materials (Harmon and Otter, 2022). Nevertheless, recent studies have established a relationship between human exposure to this plasticizer and the development of fatty liver disease and obesogenic effects (Zhang et al., 2023). Isophorone diisocyanate (IPDI) is another plastic additive mainly used in the production of polyurethane coatings (Arsenio et al., 2023). According to European Chemical Agency, it is reported their long-term toxic effects in aquatic life and their relationship with skin and respiratory irritations (ECHA, 2023).

The main goals of this study are the characterization of different food packaging materials and the identification of different substances associated with the plastic polymers.

2. Materials and Methods:
Fifty-eight packages for different food and beverage samples were selected. Foodstuffs include sliced cheese, bread, breakfast cereals, fruit, chocolate, sweeteners, infant food, sunflower seeds, toasted bread, tea, coffee, yogurt, ready-to-cook chickpeas, cola drinks, hummus, lettuce, pasta, potatoes, ready-to-cook pizza, meat, Spanish omelet, butter, wine cork, caramel, chewing gum, cooking bag, ready-to-cook vegetables and purée, eggs, smoked salmon and milk. In addition, paracetamol blister and plastic rings for cola cans were also included. These materials were cut with scissors and scalpels in order to perform the measurements. Characterization of polymers was carried out by ATR-FTIR spectroscopy. All acquired spectra were subjected to comparison with standard spectra sourced from commercially available databases. Regarding plastic additives screening, 2 mg of each sample were weighted in a metallic sample cap, based on previous methodologies (Akoueson et al., 2021). Two isotopically labeled standards, di(2-ethylhexyl) phthalate (DEHP-d4) and dibutyl phthalate (DBP-d4) were added so as to check the analysis performance by thermal desorption coupled to GC-MS. Ionization mode was electron impact, while acquisition mode was full scan between 50 and 600 m/z. Peaks were identified by comparing to NIST database, establishing a match threshold of 70%.

3. Results:
Among all the packages examined in this study, PE/PET were the most frequently identified polymers with 29 samples (50%). PP was the next most commonly detected polymer, occurring in 14 of the samples (24%), while PS and PVC were present in 4 and 3 samples, respectively. Additionally, 8 samples were included in the group “Other polymers”. In this group, poly(1,4-butylene succinate) was identified in a coffee capsule, poly(cyclohexylene terephthalate) in a fruit bag, poly[ethylene:propylene]:dien in a wine cap, nylon in a layer of Spanish omelet packaging, poly[propylene:ethylene] in a layer of vegetable purée, poly[acrylic acid]:ethylene] [surlon] in two breakfast cereal bags, and a phenoxy/epoxy resin in a cola aluminum can. Regarding GC-MS screening, Table 1 present the results for the most frequently detected compounds. ATBC was found in 37 samples (64%), which is equivalent to the number of samples containing tris(2,4-ditert-butylphenyl) phosphate. Nonanal (23 samples, 40%),...
2-(2-hydroxypropoxy)-1-propanol (14 samples, 24%) and 2,4-di-tert-butylphenol (12 samples, 21%) were also commonly found. It is remarkable the presence of isophorone diisocyanate in 8 samples, some of them infant foodstuffs and breakfast cereals.

Table 1: Tentatively identified compounds in different packaging materials

<table>
<thead>
<tr>
<th>Compound name</th>
<th>Formula</th>
<th>PE/PET (n=29)</th>
<th>PP (n=14)</th>
<th>PVC (n=3)</th>
<th>PS (n=4)</th>
<th>OTHERS (n=8)</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-(2-hydroxypropoxy)-1-propanol</td>
<td>C₆H₁₀O₃</td>
<td>9</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Isophorone diisocyanate (IPDI)</td>
<td>C₁₂H₁₆N₂O₂</td>
<td>6</td>
<td>1</td>
<td></td>
<td></td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Tributyl acetylcitrate (ATBC)</td>
<td>C₁₇H₃₄O₈</td>
<td>17</td>
<td>10</td>
<td>3</td>
<td>1</td>
<td>6</td>
<td>37</td>
</tr>
<tr>
<td>Benzene, 1,1'-methylenebis[4-isocyanato-]</td>
<td>C₁₆H₁₄N₂O₂</td>
<td>9</td>
<td>2</td>
<td></td>
<td></td>
<td>11</td>
<td></td>
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<tr>
<td>Nonanamide</td>
<td>C₁₃H₂₆NO</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td></td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Hexanedioic acid, bis(2-ethylhexyl) ester</td>
<td>C₁₄H₂₀O₄</td>
<td>4</td>
<td></td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>1,4-benzenedicarboxylic acid, 2-hydroxyethyl methyl ester</td>
<td>C₁₅H₁₂O₂</td>
<td>7</td>
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<td>2-Propanol, 1,1'-oxybis-</td>
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<td>5</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Octadecanamide</td>
<td>C₁₆H₃₂NO</td>
<td>6</td>
<td>2</td>
<td>1</td>
<td></td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Tris(2,4-di-tet-butylphenyl) phosphite</td>
<td>C₂₃H₃₆O₇P</td>
<td>15</td>
<td>12</td>
<td>1</td>
<td>2</td>
<td>7</td>
<td>37</td>
</tr>
<tr>
<td>5-Hydroxymethylfurfural</td>
<td>C₇H₁₀O₂</td>
<td>3</td>
<td>1</td>
<td></td>
<td>1</td>
<td>5</td>
<td></td>
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<tr>
<td>Benzoic acid, 2-benzyol-, methyl ester</td>
<td>C₁₅H₁₂O₂</td>
<td>3</td>
<td>1</td>
<td></td>
<td></td>
<td>4</td>
<td></td>
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<tr>
<td>Benzene, 2,4-diisocyanato-1-methyl-</td>
<td>C₁₅H₁₄N₂O₂</td>
<td>5</td>
<td>2</td>
<td></td>
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<td>1</td>
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<tr>
<td>Nonanal</td>
<td>C₉H₁₈O₂</td>
<td>9</td>
<td>4</td>
<td>3</td>
<td>7</td>
<td>23</td>
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<td>1-Propene-1,2,3-tricarboxylic acid, tributyl ester</td>
<td>C₁₄H₁₅O₃</td>
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<td>2</td>
<td>1</td>
<td>6</td>
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<td>Butyl citrate</td>
<td>C₈H₁₀O₂</td>
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<td>1</td>
<td>2</td>
<td></td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>2,4-Di-tet-butylphenol</td>
<td>C₁₄H₁₅O₃</td>
<td>5</td>
<td>5</td>
<td></td>
<td>2</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Di-n-octyl phthalate</td>
<td>C₁₄H₂₈O₄</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td>2</td>
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</tr>
<tr>
<td>Tris(2,4-di-tet-butylphenyl) phosphate</td>
<td>C₂₃H₃₆O₇P</td>
<td>3</td>
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<td></td>
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<td>Pyridine, 4-(1-pyrrolidinyl)</td>
<td>C₉H₁₂N₂</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td>4</td>
<td></td>
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<tr>
<td>Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-</td>
<td>C₂₃H₃₆O₇P</td>
<td>7</td>
<td></td>
<td></td>
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<tr>
<td>hydroxy-, octadecyl ester</td>
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<tr>
<td>Diocyl terephthalate</td>
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<td></td>
<td>1</td>
<td></td>
<td>3</td>
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<td>Phthalic acid, di(oct-3-yl) ester</td>
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<td>1</td>
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<td>n-Butyl methacrylate</td>
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<td>Octadecanenitrile</td>
<td>C₁₈H₃₄N</td>
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<td></td>
<td>1</td>
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<td>4</td>
<td></td>
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<tr>
<td>Isophthalic acid, di(2-fluorophenyl) ester</td>
<td>C₁₈H₁₄F₂O₄</td>
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<td>2</td>
<td></td>
<td>1</td>
<td>1</td>
<td>5</td>
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</tbody>
</table>
THU-PM2-D1 Integrated LC-MS/MS Analysis of 47 Per- and Polyfluoroalkyl Substances in Food Contact Materials

4. Discussion:
Results found in this study showed the predominant utilization of ATBC in food packaging, as it was legislated by European Union (EU) (Zhang et al., 2023). This trend signifies the increasing substitution of conventional phthalates with ATBC, and it agreed with the observed presence of this plasticizer in different foodstuffs (Zhang et al., 2022). Despite its established safety profile (Harmon and Otter, 2022), an increasing body of evidence suggests its detrimental effects on metabolic processes. Of particular concern is the more recent identification of ATBC-induced impairment in the generation of neurons (ASBMB Today, 2023). The other additive also commonly found, tris(2,4-di-tert-butylphenyl) phosphite, is an antioxidant which ingestion undergoes metabolic transformation into 2,4-di-tert-butylphenol (Parris et al., 2020). This compound, also found in 21% of
samples, favors lipid accumulation and behaves as an endocrine disruptor (Ren et al., 2023), highlighting a potential concern associated with its usage. It must be noted the presence of diisocyanate compounds in baby formula and infant food samples. These are additives used with the purpose of expand polymer molecular chains. However, their use has started to be under scrutiny, with recent evidences of their ecotoxicity (Li et al., 2023).

Some of the detected compounds can be encompassed in the group of non-intentionally added substances (NIAS), such as nonanal, 2-(2-hydroxypropoxy)-1-propanol, octadecanamide, acetophenone, or methyl stearate. It seems PE/PET samples showed higher presence of NIAS than other polymers, which is in accordance with other studies (Kim et al., 2023).

5. Conclusions:
Among the screened samples, PE/PET packaging emerged as the predominant choice, thereby exhibiting the highest prevalence of additives and NIAS. The gradual substitution of traditional plasticizers with ATBC has resulted in a reduced presence of phthalates. The diverse range of antioxidants and other plastic additives present in food packaging have the potential to migrate into foodstuffs through contact or by cooking, emphasizing the issue of plastic contamination. Future research efforts should prioritize investigating the impact of these factors on the human diet.

6. Acknowledgments:
This study was supported by the Spanish Ministry of Science and Innovation (Project EXPOPLAS (PID2019-110576RB-I00), Grants CEX2018-000794-S and PRE2020-093018 funded by MCIN/AEI/10.13039/501100011033 and by "ESF Investing in your future"), and by the Generalitat de Catalunya (Consolidated Research Group 2021 SGR01150).

7. References:
THU-PM2-D1  Integrated LC-MS/MS Analysis of 47 Per- and Polyfluoroalkyl Substances in Food Contact Materials


Advances in the (Bio)Remediation of POPs

P-001  Detection, quantification, and treatment of per- and polyfluoroalkyl substances (PFAS) in groundwater (DFEAT-PFAS)

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Introduction: Over the past century, a range of synthetic compounds have been produced to improve humanity’s quality of life. These include pharmaceuticals, plastics, and other chemical compounds that possess properties making them potentially harmful when released to the environment (e.g., ecological and health impacts to humans and animals). Per- and polyfluoroalkyl substances (PFAS) are a large group of chemicals used in the formulations of thousands of consumer goods, including aqueous film-forming foams used to suppress aviation fires in training scenarios, non-stick cookware, fast-food wrappers, water-repellent fabrics, medical equipment, and plastic and leather products. Because of the recent regulations and restrictions on the use of long chain (>C8) PFAS a significant shift in the industry towards short (C4-C7) and ultrashort (C1-C3) chain alternatives has been recognized the last years. Due to the high polarity and water solubility of ultrashort PFAS, the potential for bioaccumulation is low. However, the high persistence of ultrashort-chain PFAS will result in environmental accumulation, especially in aquatic environments, leading to potential risks for aquatic organisms and increased human external exposure through drinking water. Ultrashort PFAS like trifluoroacetic acid (TFA) are low to moderately toxic to a range of organisms. In addition, ultrashort PFAS can penetrate natural and anthropogenic barriers and eventually reach drinking water sources. Because common drinking water treatment techniques do not sufficiently remove them, they may reach human consumption (Jiao et al. 2022)

Materials and Methods: In the project we are focusing on detecting and removing PFAS, especially ultrashort-chain PFAS from contaminated groundwater. We are designing passive sampling devices, which can collect and monitor the temporal profile of PFAS species in groundwater. This will allow us to analyze PFAS contaminations in German and Israeli groundwater using state-of-the-art and novel analytical techniques and understand the extent of contamination. In addition to quantification, PFAS contaminated groundwater will be treated via a two-stage process to produce PFAS-free drinking water.

Results: As ultrashort-chain PFAS are difficult to analyze with the current target (LC-MS/MS) and sum parameter (AOF, EOF) analysis methods, we additionally using gas chromatography – mass spectrometry (GC-MS). Therefore, an analytical method based on GC-MS is in development to analyze the volatile ultrashort-chain PFAS (TFA, PFPrA, TFMS, PFEtS, PFPrS, trifluoroethanol, pentafluoropropanol and hexafluoro isopropanol) directly in contaminated groundwater samples with the headspace technique and in eluates of organic solvents from the developed passive sampler after direct injection. Moreover, a two-stages process is designed to increase the low concentrations found in groundwater using novel membranes processes such as closed-circuit reverse osmosis (CCRO) and mixed matrix composite nanofiltration membranes (MMCM). Next, the rejected streams containing higher concentrations of PFAS will be treated by coagulation, and the remaining PFAS adsorbed onto carbonaceous nanomaterials (CNMs).

Discussion and Conclusion: The DEFEAT-PFAS project will result in the development of novel tools to detect, quantify, and remove PFAS, especially ultrashort-chain PFAS from contaminated groundwater, and will acquire a new understanding of the extent of these contaminations.

Acknowledgments: The authors thank the German Federal Ministry of Education and Research (BMBF; 02WIL1660A and 02WIL1660B) and the Israeli Ministry of Science and Technology (MOST) for funding within the German-Israeli Water Technology Cooperation Program.

References:
Advances in the (Bio)Remediation of POPs

P-002 Combined Methods of Ion Exchange and UV/sulfite Degradation for Removal of Per- and Polyfluoroalkyl Substances (PFAS)

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Introduction: Over the past few decades, several reduction and oxidation methods have been developed for the degradation of per- and polyfluoroalkyl substances (PFAS) in water. One promising technique is the use of advanced reduction processes (ARPs) with a UV/sulfite system, which have shown high degradation and defluorination efficiency for PFAS. However, the typical concentration of PFAS reported in contaminated water is usually too low (ng/L to µg/L) to be directly treated with a UV/sulfite system, making it uneconomic in most situations. Therefore, pre-treatment methods that concentrate PFAS are necessary before applying the UV/sulfite system. As the sorption/desorption process has been successfully applied for PFAS enrichment from contaminated water, the combination of sorption/desorption and a UV/sulfite system for PFAS removal could provide a feasible solution for environmental remediation. This study provides insights into the following aspects: (1) structure-dependent sorption mechanisms based on quantum computing; (2) the regeneration efficiencies and desorption mechanisms of selected AERs using various types of desorption solutions; (3) the feasibility of using a UV/sulfite system to treat concentrated PFAS in different desorption eluents.

Materials and Methods: This study aimed to investigate the efficacy of a combination of sorption/desorption and photocatalytic reduction processes for the removal of PFAS. Specifically, PFPrA, PFHxA, PFOA, PFOS, and GenX were chosen as target PFAS and the suitability of Purolite A532E, A600, and A860 was assessed for the adsorption/desorption of these PFAS. Various types of desorption solutions (NaCl, NH₄Cl, NaOH and ethanol) were studied for resin regeneration. Subsequently, a UV/sulfite system was applied to treat the desorption effluent. Besides, the sorption/desorption mechanisms of different PFAS were analyzed based on quantum computing.

Results: Table 1: The recovery (%) of PFPrA, PFHxA, PFOA, PFOS and GenX by different desorption solution (resin and dosage: 1 g/L of Purolite® A860, PFAS concentration in adsorption processes: 50 mg/L, desorption time: 24 h, temperature: 21 ∘C, experiments were done in batch mode).

<table>
<thead>
<tr>
<th>Desorption solution</th>
<th>PFAS</th>
<th>20% EtOH</th>
<th>5% NaCl</th>
<th>5% NaCl + 20% EtOH</th>
<th>0.025% NaOH</th>
<th>0.025% NaOH + 20% EtOH</th>
<th>5% NH₄Cl</th>
<th>5% NH₄Cl + 20% EtOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFPrA</td>
<td>2.50%</td>
<td>58.4%</td>
<td>62.1%</td>
<td>56.4%</td>
<td>60.8%</td>
<td>82.8%</td>
<td>82.7%</td>
<td></td>
</tr>
<tr>
<td>PFHxA</td>
<td>0.40%</td>
<td>79.0%</td>
<td>84.5%</td>
<td>82.0%</td>
<td>75.5%</td>
<td>87.0%</td>
<td>81.1%</td>
<td></td>
</tr>
<tr>
<td>PFOA</td>
<td>0.63%</td>
<td>0.62%</td>
<td>0.66%</td>
<td>15.9%</td>
<td>22.3%</td>
<td>79.5%</td>
<td>80.3%</td>
<td></td>
</tr>
<tr>
<td>PFOS</td>
<td>0.02%</td>
<td>0.04%</td>
<td>14.8%</td>
<td>0.44%</td>
<td>1.48%</td>
<td>0.62%</td>
<td>6.58%</td>
<td></td>
</tr>
<tr>
<td>GenX</td>
<td>0.67%</td>
<td>67.7%</td>
<td>74.8%</td>
<td>61.9%</td>
<td>83.2%</td>
<td>82.2%</td>
<td>87.0%</td>
<td></td>
</tr>
</tbody>
</table>

Discussion and Conclusion: The results revealed that the addition of 20% EtOH alone was not effective in desorbing any of the PFAS, while the addition of 20% EtOH in brine resulted in increased recovery for most PFAS, possibly due to the weakened hydrophobic interactions. The combination of NH₄Cl and EtOH as the desorption solution generally showed high desorption efficiency for most PFAS species. The desorption of PFOS proved to be the most challenging, with the highest desorption efficiency only reaching 14.8% when using 5% NaCl + 20% EtOH. The results of UV/sulfite degradation showed that direct degradation of PFPrA, PFHxA, PFOA and GenX in the desorption solution (NaOH, NaCl and NH₄Cl) is effective. However, the addition of EtOH in the brine reduced the degradation and defluorination efficiency, which suggests the EtOH should be separated from water before the degradation, or the addition of EtOH need to be avoided in practical application.

Acknowledgments: This research work is conducted under the CORE-PFAS project (Combined Ion Exchange-Reduction Process for the Elimination of Fluorinated Contaminants in Waters), funded by the Academy of Finland.
Advances in the (Bio)Remediation of POPs

P-003  Inducible expression of organic pollutant transporter genes in Cucurbitaceae family by agrochemicals

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Introduction: Persistent organic pollutants (POPs) and polycyclic aromatic hydrocarbons (PAHs) with chemical persistency, high bioaccumulation, and long distance mobility remain in the wide environment for a long time. These adversely affect human health by the intake of contaminated foods. It is reported that organochlorine pesticides, such as dieldrin, which was used as a pesticide in the past, and unintentionally produced PAHs were detected in crops. Therefore, it is essential to remove them from agricultural environment. Most plants cannot accumulate hydrophobic organic pollutants in the aerial parts, but the Cucurbitaceae family, especially zucchini plants, accumulates them in the aerial parts at high concentrations¹. In Cucurbitaceae family, major latex-like proteins (MLPs) act as an organic pollutant transporter². The treatment of the fungicide Daconil to zucchini plants reduced the contamination of hydrophobic organic pollutants through the downregulation of MLP genes³. Conversely, if we can find compounds that induce MLP gene expression and increase the amount of MLPs, more effective phytoremediation using Cucurbitaceae family will be achieved. In this study, we searched for compounds that induce the expression of MLP genes in zucchini plants from agrochemicals and aimed to use these compounds for soil remediation of organic pollutants.

Materials and Methods: We selected the fungicide Oryzemate containing probenazole as an active ingredient because probenazole induces gene expression of pathogenesis-related protein class 10 (PR-10)⁴, which has a similar 3D structure to MLPs. Firstly, β-glucuronidase (GUS) activities in roots of the transgenic tobacco plants expressing the GUS gene under the control of the MLP promoters were measured after treatment with probenazole. Next, zucchini plants were cultivated in the soil contaminated with pyrene, a kind of PAHs. Pyrene concentration in the xylem sap was measured, and western blot analyses of root proteins and xylem sap were performed using anti-MLP antibodies. Then, the binding ability of probenazole to MLPs was evaluated using a competitive binding assay.

Results: GUS activity significantly increased in the transgenic tobacco plants after treatment with probenazole. When zucchini plants were cultivated in pyrene-contaminated soil at 1.25 mmol/kg and treated with Oryzemate, pyrene concentration in the xylem sap increased depending on the amount of Oryzemate. Then, band intensity of MLPs at 17 kDa significantly increased by Oryzemate in the western blot analyses of root proteins and xylem sap. In contrast, when zucchini plants were cultivated in pyrene-contaminated soil at 0.125 mmol/kg and treated with Oryzemate, pyrene concentration in the xylem sap significantly decreased. To investigate the cause, a competitive binding assay was performed. It showed that probenazole inhibited the binding of pyrene to MLPs.

Discussion and Conclusion: Probenazole induced the expression of MLP genes, resulting in the increase of MLPs in roots and xylem sap. Thus, it is proposed that the amount of pyrene transported by MLPs is increased. However, in low concentration of pyrene in soil, pyrene concentration in xylem sap was significantly decreased by Oryzemate. MLPs bind to compounds with an indole-like structure, such as amisulbrom⁵, and then pyrene concentration in xylem sap was competitively decreased by amisulbrom. Since probenazole also has such structure, it inhibits formation of pyrene-MLP complex, resulting in the decrease of pyrene concentration in the xylem sap. This study provides insight into the regulation of crop contamination by organic pollutants.

References:
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P-004 Clarification of physiological functions of transporting factors for persistent organic pollutants in Cucurbitaceae family

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Introduction: Organic pollutants are widely distributed in the world and contaminate the environment. Of those, persistent organic pollutants (POPs) show the persistency, long-distance transport, and high bioaccumulation. POPs bring harmful effects to humans, such as teratogenicity, immunotoxicity, reproductive toxicity, and carcinogenicity, by the intake of such contaminated crops. Generally, plants do not accumulate POPs in their aerial parts, like stems, leaves, and fruits. However, high concentrations of POPs are detected in the aerial parts of the Cucurbitaceae family, such as cucumber, winter squash, and zucchini. This uptake mechanism is roughly divided into 3 steps; solubilization of POPs from soil organic matters by organic acids exudated from roots, absorption of POPs to roots, and transport from roots to the aerial parts. In Cucurbitaceae family, the transport of POPs from the roots to xylem vessels plays a crucial role in their uptake¹. Since xylem sap is a solution like water, it cannot dissolve hydrophobic substances. However, POPs are dissolved in the xylem saps of Cucurbitaceae plants. This indicates that Cucurbitaceae plants have the substances that can solubilize POPs. The proteins related to solubilization were identified as major latex-like proteins (MLPs)². MLPs produced in the root cells bind to various POPs through their internal hydrophobic cavity, resulting in increase of their water solubility³. Pathogenesis-related protein class10 (PR-10) has the similar 3D structure to MLPs and binds hormones, such as cytokinins. Furthermore, PR-10 is involved in defense responses against pathogen attacks. Therefore, it is assumed that MLPs have similar functions, but physiological functions are not clear yet. In this study, physiological functions of MLPs in Cucurbitaceae family are clarified.

Materials and Methods: The zucchini seedlings were incubated in hydroponics containing cytokinins. The expression level of MLP genes in seedling roots were determined by qRT-PCR. Docking models of MLPs and various cytokinins were prepared by AutoDock Vina, and binding free energies were estimated. Also, we performed the in vitro binding experiments using recombinant MLPs and cytokinins attached to magnetic beads. The transgenic tobacco plants overexpressing MLP genes were cultivated on agar medium containing cytokinins to observe phenotypic changes.

Results: The expression levels of MLP-PG1 and MLP-GR3 genes were significantly increased 6 hours after cytokinin treatment. Docking models of MLPs and cytokinins suggest that MLPs can bind cytokinins. Furthermore, in vitro binding experiments showed that the band intensity using cytokinin-binding beads was stronger than that using control beads without cytokinins. Therefore, it is indicated that MLP-PG1 and MLP-GR3 can bind cytokinins. The transgenic tobacco plants expressing MLP genes and vector control plants (VC) exhibited phenotypic alterations, such as short and thick roots, and axillary buds formation when incubated on media containing cytokinins. Also, the fresh weights of aerial parts in transgenic tobacco plants were higher than those in VC.

Discussion and Conclusion: MLP gene promotors have the cytokinin responsive cis-elements⁴. Thus, it is thought that the upregulation of MLP genes by the treatment with cytokinins is caused by the transcriptional regulation of MLP genes through these cis-elements. Furthermore, the fresh weights in aerial parts of transgenic tobacco plants were dependent on cytokinin concentrations. It may be caused by the enhancement of cytokinin transport from roots to aerial parts. Therefore, when zucchini plants receive some stresses at roots, the MLP-cytokinin complexes are transported from roots to aerial parts to respond and resist stresses.

References:
**Introduction:** Persistent organic pollutants (POPs) cause environmental pollution on a global scale, due to their low-degradability, long-range mobility, and bioaccumulation. POPs in the natural environment accumulate in living organisms, and finally, there are known to exhibit toxic effects on humans. In the previous study, above-ground dieldrin accumulation was found in Cucurbitaceae family, with particularly high concentrations in zucchini plants (Cucurbita pepo). In xylem sap of zucchini plants, the existence of a protein called major latex-like protein (MLP) with approximately 17 kDa was identified as the factor for this phenomenon (Inui et al., 2013). The mechanisms of POP contamination in zucchini plants can be divided into four steps (Goto et al., 2019). First, POPs are taken up by root tissues of zucchini plants. Next, MLPs bind to POPs and form MLP-POP complex in root cells. And then, the complex is secreted into the xylem sap. Finally, the complex is transported to the above-ground parts, such as stems, leaves, and fruits, through xylem sap. Since the xylem sap is a solution filled in the intercellular space that does not have the ability to synthesize proteins, MLPs must be secreted from root cells into the intercellular space called apoplast. However, MLPs do not have a signal sequence in its amino acid sequence, which is an N-terminal feature of normal secretory proteins. Therefore, MLPs are possible to be secreted into the apoplast via an unconventional secretory pathway, but these mechanisms remain to be unclear. To elucidate the mechanisms of MLP translocation from root cells to xylem vessel in zucchini plants, this study examined the localization of MLPs in the apoplast washing fluid (AWF) and in cells using roots of two zucchini cultivars with different POP accumulation abilities.

**Materials and Methods:** Roots and xylem sap were collected from two zucchini cultivars (low and high POP accumulators) grown in soil for 3 weeks. After infiltrating the roots of both cultivars with a buffer, AWF was collected by centrifugation. Besides, the roots of both cultivars were ground, and the supernatant was collected by ultracentrifugation as the cytosolic fraction and the precipitate as the microsomal fraction. The microsomal fraction was further separated into 10 fractions by a sucrose density gradient centrifugation. Western blot analysis was employed to compare the band patterns of MLPs and membrane marker proteins in each fraction. The localization of MLPs in the membrane was estimated comparing with that of membrane proteins.

**Results:** Western blot analysis revealed that the intensity of MLP-derived bands in the root AWFs of low POP accumulator was lower than that of high accumulator. From cellular fractionation experiments, MLPs were present in the cytosolic fraction in the low POP accumulator, while MLPs were absent in the high accumulator. Furthermore, the localization of MLPs in the microsomal fraction was inferred to be at the endoplasmic reticulum membrane in both cultivars.

**Discussion and Conclusion:** MLPs are not detected in xylem sap of the low accumulator but detected in the high accumulator (Goto et al., 2019). The localization of MLPs in AWF was consistent with that in xylem sap, suggesting that the extent of intercellular secretion of MLPs in root tissues is related to the MLP amount transferred to the xylem vessel. Also, there is the large difference in MLPs between the localization in the cytosolic fraction and the xylem sap. This result indicates that localization of MLPs in the cytosol is important for intercellular secretion. The clarification of MLP-mediated POP transport mechanisms in zucchini plants provides important insights for the development of technologies to control POP contamination in cucurbits.

**References:**
Advances in the (Bio)Remediation of POPs

P-007  Comparison of Ozonation and Advanced Oxidation Processes for Removal of Antibiotics in Real Wastewaters

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Introduction: The activated sludge process is the most widespread wastewater treatment method; however, it is difficult to remove antimicrobial agents, which are persistent and non-biodegradable, and remain in the discharged water. Residual antimicrobial agents in the aquatic environment can cause the development of Antimicrobial Resistance bacteria (AMR). The number of annual deaths caused by AMR is expected to increase to 10 million by 2050 if no action is taken. Ozonation is a promising method for removing non-biodegradable organic pollutants due to the feasibility, accessibility, and high reactivity of ozone; however, it is difficult to remove and mineralize the pollutants completely. To address the drawbacks, advanced oxidation processes (AOPs) have been attracting attention. In general, many previous studies have focused on a single treatment technology, and there are only a limited number of studies that comprehensively compare various AOPs. In addition, available studies are not likely to consider the effects of water matrices in actual wastewater. Thus, more comprehensive research is required. In this study, to select an efficient wastewater treatment method, we compared the removal performance of antibiotics by oxidative degradation technologies, namely ozonation, ozone/hydrogen peroxide process, and photo-Fenton reaction. In addition, we evaluated the effects of water matrices in actual wastewaters on the removal performances.

Materials and Methods: Ozonation was started by blowing 0.55 mg L−1 of ozone gas into the antibiotics aqueous solution at a gas flow rate of 3.0 L min−1. In the ozone/hydrogen peroxide method, the experiment was started by adding hydrogen peroxide (100 mg L−1). The photo-Fenton reaction was carried out by adding antibiotics, iron (II) sulfate heptahydrate (3.0 mg L−1), and hydrogen peroxide (100 mg L−1) to an aqueous solution adjusted to pH = 3.0. The experiment was started by turning three black light blue lamps (maximum irradiation wavelength = 380 nm). The solution was sampled, and the concentration of antibiotics was measured using a liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS).

Results and Discussion: Figure 1 shows the experimental results of comparing removal performances in five real wastewaters of seven antimicrobial agents by three different oxidation processes in terms of inhibition factors. In the case of ozonation and ozone/hydrogen peroxide, removal of some antimicrobial agents was enhanced in actual wastewaters, while degradation was inhibited in the case of the photo-Fenton reaction. In general, the decomposition in real wastewater tends to be inhibited by water matrices compared with that in pure water. However, the degradation in the actual sewage could be accelerated by the presence of metal ions as water matrices, which facilitate the generation of oxidizing species such as OH radicals. In conclusion, the removal performances of antibiotics by oxidation process could be significantly affected by not only the types of contaminants and oxidation processes but also water matrices in wastewater.

Acknowledgments: The project was supported by the Ministry of the Environment Research and Technology Development Fund (5-1954), Kurita Water and Environment Foundation (18A070 21A028), and Steel Foundation for Environmental Protection Technology (C-40-18).

Figure 1: Comparison of removal performances in terms of inhibitory factor.
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P-008 Dioxin removal mechanism during smoldering remediation of contaminated soil

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Introduction: The smoldering remediation technology of organic contaminated soil has significant advantages due to its high pollutant removal efficiency, self-maintenance of reaction process and low carbon emission[1, 2]. It is suitable for the remediation of organic contaminated soil such as dioxin (PCDD/Fs), perfluorinated and polyfluoroalkyl substances (PFAS) with high boiling point and difficult to degrade[3]. However, the dioxin removal mechanism as well as the influencing factors of pollutants during the smoldering remediation of dioxin contaminated soil remain to be studied.

Materials and Methods: Two dioxin contaminated soil were collected from a contaminated site of a retired municipal waste incinerator in China. The soil samples were sealed in light and stored in cold storage. Before the experiment, the contaminated soil broke over a 2mm sieve, added 8% carbon black and 8% wood chips as auxiliary fuel, and mixed evenly with the contaminated soil. The smoldering repair experimental device of contaminated soil is adopted. During the experiment, each smoldering reaction chamber is filled with 5 kg of reaction material, and the air volume of the west is 1.5 m3/h. Dioxin in the soil before and after the smoldering experiments were collected to conduct the dioxin analysis using EPA1613 method.

Results: The peak temperature of the soil during the smoldering remediation process is 500-600 ∘C, which is higher than the dioxins boiling point. The concentrations of dioxins in the contaminated soil were 235 and 457 ng/kg, respectively. The values decreased to 40 and 59 ng/kg, with the degradation rates were 83% and 87%, respectively. The I-TEQ values of dioxins in the contaminated soil were 16 and 29 ng I-TEQ/kg, respectively. The values decreased to 5 and 8 ng I-TEQ/kg, with the degradation rates were 67% and 73%, respectively. The I-TEQ values of the soil after the smoldering were both lower than 10 ng I-TEQ/kg, indicating that smoldering is a green and low-carbon remediation technology for dioxin contaminated soil.

Discussion and Conclusion: The removal rate of dioxins of contaminated soil after smoldering is higher than 80%, and the concentration of toxic equivalent is lower than the screening value of the first type of construction land. Desorption and oxidative degradation reaction are possibly the main removal paths of dioxins in contaminated soil. Smoldering is a technology suitable for green, low-carbon and sustainable remediation of dioxin contaminated soil. However, the methods to enhance the high thermal stability of smoldering process when the soil was not well treated should be studied.

Acknowledgments: Natural Sciences Fund of Zhejiang Province (LY23E060002).

References:
1. Introduction:
The filter-dust (fly ash) produced by waste-to-energy plants is classified as hazardous waste according to European law. Depending on the process conditions, a significant concentration of dioxins can be found in this type of filter-dust. However, advancements in technology in recent decades have allowed the development of effective pollution control technologies. When applied to modern waste incinerators, also known as waste-to-energy plants, these technologies ensure that practically no dioxins are released into the surrounding environment. It’s important to note, though, that dioxins can still be generated during the process of waste incineration. Depending on the specific combustion conditions, the chlorine content of the waste, the presence of metals like copper that can catalyse the formation of dioxins, and even improper delivery of unsuitable materials for incineration, a significant concentration of dioxins can be found in the filter dust. However, in modern waste-to-energy plants, effective abatement systems safely capture and retain dioxins, ultimately leading them to accumulate in the filter dust. As a precautionary measure, European waste regulations classify the residues generated from flue gas treatment as dangerous, assigning them the CER code 190105* (dangerous). Consequently, this material cannot be recovered but must be disposed of in specialized and secure landfill sites. However, if a treatment method is applied to the filter dust that demonstrates the complete elimination of dioxins, regardless of their initial concentration, the resulting product can be considered for material recovery operations. From a sustainability standpoint, it is crucial to move towards a circular economy, where everything is reused, and waste is minimized. This is the path we should pursue in the coming decades if we aim to preserve a liveable environment for future generations, ensuring a healthy planet for our grandchildren. In the past, farmers practiced a mindset of waste elimination, and we too should strive to adopt similar practices. Transitioning towards a zero-waste lifestyle model is necessary for a more sustainable future.

If we continue to generate waste that ends up in landfills, we are essentially passing on the problems to future generations. To address this issue, Eco Research has developed a decontamination technique based on the hydrothermal decarbonization (HTC) reaction. Hydrothermal carbonization (HTC) is a thermo-chemical process used for converting biomass. The process mimics the natural process of coal formation. Solid and wet biomass is dehydrated and transformed into bio-coal with a heating value similar to brown coal, typically achieved at temperatures around 200 °C and pressures of about 20 bar. This process was first described by Friedrich Bergius in the early 20th century while studying the formation of naturally occurring coal.

Under HTC conditions, specifically at temperatures above 300 °C, there is an almost complete removal of all polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (PCDD/Fs) from fly ash. The HTC process allows for the total destruction of dioxins at relatively lower temperatures compared to other techniques, and it takes place in an aqueous environment. Following treatment, the filter-dust can be reclassified as a recoverable material, offering the potential for resource recovery and reuse.

2. Materials and Methods:
Near the city of Bolzano, Italy, an HTC pilot plant has been constructed and put into operation for the purpose of decontaminating the filter-dust from the local waste-to-energy plant. This pilot plant is capable of operating at temperatures up to 350 °C and pressures of 150 bar. The vessel used in the process has a capacity of approximately 1 m³, allowing for the treatment of up to 200 kg of material under wet HTC conditions.

The typical operating conditions of the pilot plant involve maintaining a temperature of 310 °C and a pressure of around 100 bar for a duration of 8 hours. In order to assess the efficiency of the decontamination process, the reactor is filled with approximately 500 liters of water, and 100 kg of fly ash is mixed with biomass, such as sawdust, and brought to the required temperature for the HTC process (310 °C in the case of dioxin abatement). It is essential to continuously stir the mixture in the reactor throughout the entire treatment, which lasts approximately 8 hours. This stirring is crucial to ensure the homogeneity of the mixture of fly ash, biomass, and water. Without proper mixing, stratification and phase separation occur, resulting in an ineffective reaction. To overcome the challenges of operating under high temperatures and pressures, the plant is equipped with a magnetic transduction stirrer, as maintaining a seal on the through shaft of the stirrer becomes difficult under such conditions.

Upon completion of the reaction, the reactor undergoes a cooling process, which consequently reduces the internal pressure as well.

Once the reactor has cooled down, if necessary, a minimum pressure is maintained using compressed air to assist in the expulsion of the material at the end of the process. The obtained material is then dried in an oven at 105 °C and prepared for analysis.
For the analysis of the sample, the extraction and clean-up of tetra- to octa-chlorinated PCDD/Fs (polychlorinated dibenzo-p-dioxins and dibenzofurans) were performed following the US EPA 1613 method. The quantification of the PCDD/Fs was carried out using isotope dilution with gas chromatography coupled with a triple quadrupole mass spectrometer. Specifically, a Thermofisher TRACE GC 1300 gas chromatograph was coupled with a Thermofisher TSQ 8000 Evo mass spectrometer. Gas chromatographic separation of the analytes was achieved using a DB 5 ms (Agilent.) column, 60 m x 0.25 mm i.d. and a film thickness of 0.25 µm.

3. Results:
Table 1 presents the results of two HTC test runs conducted at a temperature of 310 °C. The table indicates that the abatement efficiency for dioxins and furans ranged between 88% and 98%, depending on the single compound. On a TEQ basis, considering the total toxicity, the abatement efficiency was 93% (Test A) and 94% (Test B). However, in order to further increase the abatement efficiency, it is recommended to optimize some operating parameters. These optimizations can potentially enhance the effectiveness of the decontamination process and lead to even higher levels of dioxin elimination.

Table 1: Abatement results obtained from two HTC treatment tests (A and B)

<table>
<thead>
<tr>
<th>Congener</th>
<th>TEST: A</th>
<th></th>
<th></th>
<th></th>
<th>TEST: B</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before HTC</td>
<td>After HTC</td>
<td>Abatement</td>
<td>Before HTC</td>
<td>After HTC</td>
<td>Abatement</td>
<td></td>
</tr>
<tr>
<td>Results in ng/kg</td>
<td>in %</td>
<td>Results in ng/kg</td>
<td>in %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2378 TCDD</td>
<td>18</td>
<td>1,7</td>
<td>91</td>
<td>18</td>
<td>0,8</td>
<td>96</td>
<td></td>
</tr>
<tr>
<td>12378 PCDD</td>
<td>44</td>
<td>5,4</td>
<td>88</td>
<td>41</td>
<td>4,7</td>
<td>89</td>
<td></td>
</tr>
<tr>
<td>123478 HxCDD</td>
<td>32</td>
<td>2,2</td>
<td>93</td>
<td>24</td>
<td>2,4</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>123678 HxCDD</td>
<td>54</td>
<td>4,7</td>
<td>91</td>
<td>61</td>
<td>6,8</td>
<td>89</td>
<td></td>
</tr>
<tr>
<td>123789 HxCDD</td>
<td>88</td>
<td>5,8</td>
<td>93</td>
<td>64</td>
<td>6,9</td>
<td>89</td>
<td></td>
</tr>
<tr>
<td>1234678 HpCDD</td>
<td>521</td>
<td>6,2</td>
<td>99</td>
<td>311</td>
<td>10,8</td>
<td>97</td>
<td></td>
</tr>
<tr>
<td>OCDD</td>
<td>1273</td>
<td>13,3</td>
<td>99</td>
<td>1073</td>
<td>32,5</td>
<td>97</td>
<td></td>
</tr>
<tr>
<td>2378 TCDF</td>
<td>146</td>
<td>1,6</td>
<td>99</td>
<td>168</td>
<td>5,8</td>
<td>97</td>
<td></td>
</tr>
<tr>
<td>12378 PCDF</td>
<td>137</td>
<td>4,8</td>
<td>97</td>
<td>137</td>
<td>5,4</td>
<td>96</td>
<td></td>
</tr>
<tr>
<td>23478 PCDF</td>
<td>143</td>
<td>3,3</td>
<td>98</td>
<td>129</td>
<td>5,6</td>
<td>96</td>
<td></td>
</tr>
<tr>
<td>123478 HxCDF</td>
<td>103</td>
<td>2,7</td>
<td>97</td>
<td>158</td>
<td>6,5</td>
<td>96</td>
<td></td>
</tr>
<tr>
<td>123678 HxCDF</td>
<td>123</td>
<td>3,0</td>
<td>98</td>
<td>117</td>
<td>6,2</td>
<td>95</td>
<td></td>
</tr>
<tr>
<td>234678 HxCDF</td>
<td>85</td>
<td>3,4</td>
<td>96</td>
<td>82</td>
<td>8,1</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>123789 HxCDF</td>
<td>35</td>
<td>2,8</td>
<td>92</td>
<td>37</td>
<td>3,7</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>1234678 HpCDF</td>
<td>233</td>
<td>7,9</td>
<td>97</td>
<td>210</td>
<td>11,6</td>
<td>94</td>
<td></td>
</tr>
<tr>
<td>1234789 HpCDF</td>
<td>71</td>
<td>6,7</td>
<td>90</td>
<td>34</td>
<td>1,7</td>
<td>95</td>
<td></td>
</tr>
<tr>
<td>OCDF</td>
<td>74</td>
<td>4,3</td>
<td>94</td>
<td>142</td>
<td>13,2</td>
<td>91</td>
<td></td>
</tr>
<tr>
<td>TEQ in ng/kg (WHO 2005)</td>
<td>184</td>
<td>11,1</td>
<td>94</td>
<td>179</td>
<td>12,2</td>
<td>93</td>
<td></td>
</tr>
</tbody>
</table>
4. Discussion:
The results obtained from the pilot plant scale experiments using 100 Kg of fly ash demonstrated a significant reduction in dioxin content through the HTC treatment. Laboratory tests conducted with significant smaller amounts of fly ash (only some grams) indicated a slightly higher abatement efficiency. To further enhance the abatement efficiency on a pilot plant scale, it is recommended to optimize both mechanical parameters, such as the mixing process, and chemical parameters, including pH adjustment. It is worth noting that regardless of the scale, applying the HTC process to fly ash leads to a considerable reduction in dioxin content. Furthermore, after the HTC treatment, it is possible to safely recover metals, such as zinc, that are present in the fly ash. By further optimizing the process, it is feasible to achieve complete recovery of virtually all materials contained in the fly ash.

5. Conclusions:
The hydrothermal carbonization treatment method, conducted at temperatures around 300°C, offers the potential for decontaminating various types of industrial residues, dust from thermal process purification systems, and products that have been accidentally contaminated by persistent organic substances (POPs) such as PCDD/Fs. This method is also applicable to other substances like PCBs, certain fluorinated compounds, and brominated substances. In this treatment process, the product to be treated is mixed with organic materials such as biomass, wood, or sewage sludge from municipal water treatment, along with water. The mixture is then heated in a vessel at temperatures around 300°C, under a resulting pressure of approximately 100 bars. Over the course of a few hours, this treatment leads to a reduction of over 90% of the initially present dioxins, measured in terms of toxicity equivalents.

6. Acknowledgments:
The Department of Innovation, Research and University of the Autonomous Province of Bozen-Bolzano is gratefully acknowledged for the financial support.

7. References:
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7. Tirler W, Basso A. (2013); Chemosphere 93 1464–1470
Malgorzata Warenik-Bany, Sebastian Maszewski, Szczepan Mikolajczyk, Marek Pajurek, Wojciech Pietron, Beata Furga, Magdalena Gembal, Ewelina Milczarczyk, Joanna Cebulska, Paweł Czerski

Introduction: In July and August 2022, there was a massive fish die-off in the Oder River. In order to identify the cause of this phenomenon, investigations were undertaken for a number of contaminants, including Persistent Organic Pollutants (POPs). Some of the POPs that have a negative impact on the functioning of living organisms are dioxins, PCBs, perfluoroalkyl substances and brominated flame retardants (1,2,3). Laboratory studies suggest that chemical contaminants weaken host immune defenses, making them more susceptible to epizootic infections (4). Accordingly, a pilot study was undertaken to determine the levels of these contaminants in material collected from dead fish, and if it could be the potential cause of fish die-offs and whether possible consumption of fish from the Oder River could pose a risk to human health.

Materials and Methods: The study material consisted of samples taken from the following fish species: catfish (Silurus glanis), sturgeon (Acipenser sturio), bream (Abramis brama) and bream fry, asp (Leuciscus aspius), chub (Squalius cephalus), pikeperch (Sander lucioperca). Seventeen 2,3,7,8-substituted PCDD/Fs, twelve dioxin-like -PCBs, six non dioxin-like-PCBs, eleven PBDEs and fourteen of PFAS were investigated. For the determination of PCDDs, PCDFs, dl-PCBs and ndl-PCBs, and PBDEs was used high-resolution gas chromatography coupled with high-resolution mass spectrometry (HRGC-HRMS) and for perfluorinated compounds was used liquid chromatography with tandem mass spectrometry (LC-MS/MS).

Results: Summarized data on tested contaminants occurrence in different species of fish from river Odra are presented in Table 1.

Table.1 Levels of contaminants in fish samples (X±U).

<table>
<thead>
<tr>
<th>Species</th>
<th>PCDD/F (pg WHO-TEQ/g wet weight)</th>
<th>PCDD/F/dl-PCB (ng/g wet weight)</th>
<th>ndl-PCB (µg/kg wet weight)</th>
<th>∑PBDE</th>
<th>∑ PFHxS, PFOA, PFNA, PFOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum levels</td>
<td>(2023/915/EU)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>catfish**</td>
<td>4.98±0.71</td>
<td>26.03±5.73</td>
<td>237.82±53.91</td>
<td>24.29±4.86</td>
<td>0.83±0.17</td>
</tr>
<tr>
<td>sturgeon*</td>
<td>0.12±0.02</td>
<td>0.16±0.04</td>
<td>0.47±0.11</td>
<td>0.05±0.01</td>
<td>0.52±0.10</td>
</tr>
<tr>
<td>bream***</td>
<td>0.72±0.10</td>
<td>0.95±0.21</td>
<td>6.53±1.48</td>
<td>0.47±0.09</td>
<td>2.10±0.90</td>
</tr>
<tr>
<td>bream fry</td>
<td>0.25±0.04</td>
<td>0.41±0.09</td>
<td>4.60±1.04</td>
<td>0.27±0.05</td>
<td>1.76±0.76</td>
</tr>
<tr>
<td>asp*</td>
<td>1.90±0.27</td>
<td>4.50±0.99</td>
<td>52.26±11.85</td>
<td>2.67±0.53</td>
<td>5.34±1.07</td>
</tr>
<tr>
<td>chub*</td>
<td>0.30±0.04</td>
<td>1.10±0.24</td>
<td>12.42±2.82</td>
<td>0.44±0.09</td>
<td>0.89±0.18</td>
</tr>
<tr>
<td>pikeperch***</td>
<td>0.04±0.01</td>
<td>0.12±0.03</td>
<td>1.47±0.33</td>
<td>0.11±0.02</td>
<td>2.59±0.52</td>
</tr>
</tbody>
</table>

Discussion and Conclusion: The highest levels were determined in catfish and asp. In the case of catfish, concentrations of dioxins and PCBs exceeded the highest maximum set by EC Regulation No. 2023/915 (OJ L 119, 5.5.2023, p. 103–157). The high levels of dioxins and related compounds found in catfish may have resulted from the level occupied by this species in the trophic chain (predatory fish), the lifespan of the individual, and behavior (living in deep cavities). In the other samples tested, levels ranged from 1% to 54% of the maximum limit for dioxins and from 1% to 69% for the sum of dioxins and dioxin-like polychlorinated biphenyls (2023/915/EU). Ndl-PCBs levels were also low, within the range from 1% to 42% of the maximum limit. Levels of compounds from the polybrominated diphenylethers (PBDEs) group were higher than determined in fish from the Baltic Sea and aquaculture. In 2022, the European Commission published a recommendation obliging member countries to conduct monitoring of PFAS in food and feed and introduced the limit for the sum of PFOS, PFOA, PFNA and PFHxS, differentiated according to fish species and food purpose. We observed exceeded the permissible limits only for asp. The pilot study results allow us to conclude that the tested compounds were not the direct cause of fish deaths. However, the presence of POPs in the habitat and chronic exposure to such a multitude of chemical pollutants could negatively affect living organisms in the Oder River. It is also worth noting that the angling community and consumers of freshwater fish may be specifically exposed to dioxins and related compounds, especially through the consumption of predatory fish.

Acknowledgments: Thanks to the staff of the Department of Radiobiology for their commitment to performing the study

References:
Environmental and Human Exposure to Plasticizers and Other Consumer Product Chemicals

P-013  Comparative monitoring of phthalates and alternative plasticizers (APs) in indoor dust from Korea and Malaysia

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Introduction: Phthalates have been used worldwide as plastic additives in various industrial and consumer products, such as polyvinyl chloride (PVC), food packages, personal care products, and medical devices to improve processibility, durability, and heat resistance. The global market size for plasticizers has been growing, and Asia has grown into the world's largest plasticizer market. Korea is the third-largest country in Asia in plasticizer market, after China and Japan with highly developed plasticizer-based industries in automobiles, electronics, and plastics, which comply with global regulations. Among Asian countries, Malaysia has a relatively small plasticizer-related industrial market and lower consumption volume of plasticizers. The epidemiological studies have reported adverse health effects, such as reproductive and developmental toxicity, of legacy phthalates including bis(2-ethylhexyl) phthalate (DEHP). Due to the potential risks to human health, legacy phthalates have been regulated in consumer products worldwide. Industries have introduced phthalate-based alternative plasticizers, such as di-2-ethylhexyl terephthalate (DEHTP), diisononyl phthalate (DiNP) and newly introduced APs are being used as alternatives. Earlier studies have reported indoor dust could be secondary source of semi-volatile and volatile organic compounds to human exposure. Monitoring studies on APs in the indoor environment are still needed. The aim of this study is to analyze phthalates and APs in indoor dust samples in Korea and Malaysia, investigate differences in pollution patterns between the two countries, and conduct an exposure assessment.

Materials and Methods: In this study, indoor dust samples (n=100) were collected from Korea and Malaysia in 2021 and 2022, respectively. Fourteen legacy phthalates and 22 alternative plasticizers were measured using gas chromatography (GC) coupled with tandem mass spectrometry (MS/MS).

Results: Phthalates and APs were detected in all indoor dust samples. In Korea, the total concentrations of APs were measured to be 2 to 10 times higher than those of phthalates, while in Malaysia, similar or lower levels were observed. DEHP, DEHTP, and DiNP accounted for more than 90% of the total concentrations of plasticizer in almost all indoor dust samples from both countries, implying DEHTP and DiNP have been used as major replacement for DEHP. For legacy phthalates, DEHP was predominant in indoor dust samples, followed by di-n-butyl phthalate (OnBP) and benzyl-butyphthalate (BBzP) in both countries. For APs, DEHTP, DiNP, and diisodecyl phthalate (DiDP) was dominant in indoor dust samples in both countries, implying major plasticizers used are phthalate-based plasticizers, which are similar in both counties. Among the newly introduced APs, 2,2,4-Trimethyl-1,3-pentanediol diisobutyrate (TXIB) showed a high level in Korea, while disonyl cyclohexane-1,2-dicarboxylate (DINCH) showed a high level in Malaysia. The ratio of DEHTP/DEHP was used to evaluate the degree of transition from legacy phthalates to alternative plasticizers. The average calculated ratio of dust samples of Korea was higher than Malaysia, suggesting APs are relatively widely used in products used in the Korean indoor environment. Pearson’s correlation analysis exhibited significant correlations between phthalate and AP (r=0.439, p<0.01) in indoor dust samples from Malaysia, indicating similar contamination source. Exposure assessments in the two countries showed that daily intake of plasticizers through skin absorption was higher than ingestion of dust. And infants were more exposed to plasticizers than adults, most likely due to behaviors such as hand-to-mouth habits.

Discussion and Conclusion: In this study, indoor dust samples were collected from two Asian countries and analyzed for phthalates and APs. In Korea, DEHTP was measured at highly contaminated levels compared to other countries in previous studies. The results of the relative distribution showed country-specific pollution patterns in indoor dust, which seem to be due to the difference lifestyle factors, such as consumer products used, climate, ventilation frequency. Based on the DEHTP/DEHP ratio, phthalates used in consumer products in the Korea were rapidly replaced by alternative plasticizers. Correlation analysis results suggest that phthalates and alternative plasticizers used in products vary in Korea, while phthalates and alternative plasticizers are used together in Malaysia. Our results confirm the need for comprehensive cross-border monitoring of plasticizers in indoor environmental samples. Further study is needed to help make the base for regulations on chemicals used in consumer products to reduce human exposure.

Acknowledgments: This research was supported by a grant (21162MFDS074) from Ministry of Food and Drug Safety in 2023.
Environmental and Human Exposure to Plasticizers and Other Consumer Product Chemicals

P-014 Screening of organic compounds in indoor dust with supramolecular solvents as generic sample preparation

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Introduction: Supramolecular solvents (SUPRAS) are nanostructured liquids produced from amphiphilic compounds in aqueous or hydro-organic media through self-assembly and coacervation.¹ They are a great option to replace conventional organic solvents in analytical extractions due to their physico-chemical properties: (a) mixed interaction mechanisms with organic compounds (e.g. polar, ionic, hydrogen, dispersion interactions, etc.), (b) presence of regions with a wide range of polarity in the supramolecular aggregates, (c) high number of available binding sites (high concentration of amphiphile in the range 0.1-1 mg/µL), (d) behavior as restricted access materials for polar macromolecular interferents and (e) low volatility and toxicity in comparison with conventional solvents. All these properties make them excellent candidates for generic sample treatment of indoor dust, a complex and heterogeneous matrix containing from textile and paper fibers to human or animal hair, cells and mineral components, among others. Indoor dust samples from public environments underwent SUPRAS treatment prior to LC-QTOF analysis. A wide variety of compounds were fully or tentatively identified under target, suspect and nontarget approaches.

Materials and Methods: Dust samples were collected in public buildings from Córdoba in Spain (education buildings, shops, pubs and offices). SUPRAS were made up of inverse aggregates of 1-hexanol in THF:water mixtures. Samples of 30—50 mg (sieved to 0.5 mm) were extracted with 200 µL SUPRAS. After vortex stirring and centrifugation steps, SUPRAS extracts were directly analyzed. Analysis was done by LC-QTOF- high resolution MS/MS (Bruker ELUTE UHPLC coupled to TimsTOF) equipped with an ESI source operating in positive and negative modes.

Results: Suspect and nontarget screening was carried out by auto-MSMS acquisition mode (data-dependent acquisition by isolation and fragmentation of most abundant ions) taking into account mass accuracy, isotopic pattern fit and fragmentation score against MS libraries. For target analysis, broadband CID (data-independent acquisition, comprehensive recording of all detectable precursor and product ions, independently of precursor intensity) was used and confirmation was made on the basis of retention time, mass accuracy and isotopic pattern fit of a predefined target list of parent and fragment ions. A total of 110 compounds were identified by suspect screening, 51 compounds by target analysis and 40 compounds by nontarget approaches. Only about one third of the compounds identified by suspect and nontarget screening were classified as synthetic while the rest were natural compounds, being fatty acids and derivatives the most abundant group. Regarding the identified synthetic compounds, these included phthalates, parabens, surfactants, drugs of abuse, flame retardants and plasticizers, among others.

Discussion and Conclusion: results show the potential of SUPRAS to establish simple and generic sample treatments for multi-target approaches in complex matrices, such as indoor dust. As reported by other authors, dust contains significant amounts of potentially toxic compounds and it is an important source of exposure to contaminants in indoor environments.

Acknowledgments: authors thank the funding for the project PID2020-113743RB-I00, funded by MCIN/ AEI /10.13039/501100011033.

Environmental and Human Exposure to Plasticizers and Other Consumer Product Chemicals

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Introduction: A wide array of new chemicals has been introduced during the past decades into our environment and consumer products, many being alternatives to older, persistent and often regulated compounds. Humans are exposed to these emerging chemicals via various pathways and biomonitoring is a valuable tool to assess internal exposure and evaluate potential health risks. Many of these emerging compounds are more polar and less persistent than their older counterparts, resulting in shorter half-lives and substantial variability in urinary levels. To accurately assess exposure, study design should be optimized and temporal variability characterized. The intra-class-correlation coefficient (ICC) is a statistical and non-dimensional parameter that is often used to evaluate reproducibility of repeated measurements and ranges from poor (<0.4) to excellent (≥0.75). This review aimed to collect studies describing ICCs of non-persistent organic chemicals, to discuss variation due to study design and other potential influential factors and to formulate recommendations for future biomonitoring studies (1).

Materials and Methods: Six classes of chemicals were evaluated: bisphenols, pyrethroids, parabens, phthalates, alternative plasticizers and organophosphate flame retardants. Relevant papers were searched using PubMed and Web of Science by employing a combination of keywords: “urine” with “variability OR variation OR ICC” and a specific keyword related to the chemical class or compound. Studies that collected urine samples only during one single day were excluded. The influence of urinary dilution on the variability was evaluated by including dilution correction using creatinine levels (CRT), specific gravity (SG) or osmolality (OSM). The influence of the study design was assessed by distinguishing between collection of spot samples, morning voids and/or 24 h pools.

Results: This is the first review demonstrating the influence of specific factors (i.e. study population, sampling strategy, type of urinary dilution adjustment) on the temporal variability of a wide range of short half-life chemicals. More than 60 studies were included, showing that the reported ICCs for individual chemicals varied greatly between studies (e.g. propyl paraben ICC ranging from 0.28 to 0.91). The highest ICCs were reported for parabens (median = 0.52), while 3-phenoxybenzoic acid (3-PBA) and bisphenol A (BPA) showed the lowest values (0.08 and 0.20, respectively). Most of the chemicals with ICCs <0.4 continue to show low reproducibility following correction with SG and/or CRT. The median ICCs in 24 h pooled urine samples had a tendency to be higher than those for morning void or spot urine samples.

Discussion and Conclusion: The large variation in reported ICCs for individual chemicals indicated the large impact of the study design. Chemicals for which the diet is the main exposure source (e.g. BPA, 3-PBA, metabolites of the phthalate DEHP) showed low ICCs regardless of study design or population, while contaminants with specific indoor exposure sources (e.g. metabolite of the flame retardant TDCIPP and chemicals related to personal care products such as parabens) showed higher ICCs. The exposure route is one of the most important determining factors of temporal variability. Single samples might not accurately reflect exposure to most non-persistent organic chemicals, especially when small populations are considered. Future biomonitoring studies that examine compounds showing high variability should take adequate measures to enable accurate exposure assessment, such as correction of urinary dilution or collection of multiple samples per participant.

Acknowledgments: M. Roggeman acknowledges funding through a Research Foundation Flanders (FWO) fellowship (1133223N). Y. Ait Bamai acknowledges a fellowship from the Japan Society for the Promotion of Science (JSPS) through the Fund for the Promotion of Joint International Research (Fostering Joint International Research (A), grant number 19KK0288). A. Klimowska acknowledges a fellowship (BPN/BEK/2021/1/00132/U/00001) funded by the National Agency for Academic Exchange (NAWA) under the Bekker Programme (2021).

1. Introduction:
Organophosphorus compounds are widely used as flame retardants and plasticizers in plastic products with concentrations as high as several percent order. The use of organophosphorus compounds as alternative flame retardants has increased in line with restrictions on the production and use of brominated flame retardants, such as polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCD), by the Stockholm Convention on Persistent Organic Pollutants. Organophosphorus compounds are additive-type additives and are not chemically bound to the product material, which results in migration outside the product. As a result, human is exposed to organophosphorus compounds via indoor air and indoor dust. Some organophosphorus compounds have been reported to have toxicity to humans. For example, tris (2-chloroethyl) phosphate (TCEP) has been reported to cause kidney lesions, tris(2-chloroisopropyl) phosphate (TCP) to cause periportal hepatocyte swelling and mild thyroid follicular cell hyperplasia, and tris(1,3-dichloro-2-propyl) phosphate (TDCPP) to increase relative liver weight.

Water is the most critical human resource; thus, its safety must be deeply considered for human health. Some organophosphorus compounds have moderate to high water solubility and may leach into drinking water if product components, including organophosphorus compounds, contact the water. In fact, organophosphorus compounds have been detected in tap waters and bottled waters, and drinking water could be a significant exposure route for organophosphorus compounds in humans. Water dispensers, whose market has been expanding in recent years due to the influence of COVID-19, are more specialized in the purpose of drinking compared to tap water which is also used for washing. They have a heating facility to provide hot water immediately; thus, they could contain organophosphorus compounds to prevent fire risk. However, information on the occurrence of organophosphorus compounds has been limited.

In this study, organophosphorus compounds in drinking water collected from water dispensers in Japan were measured using liquid chromatography-tandem mass spectrometry (LC-MS/MS), and screening-level exposure and human health risk assessment were carried out.

2. Materials and Methods:

**Chemicals and materials**
Trimethyl phosphate (TMP), triethyl phosphate (TEP), tributyl phosphate (TBP), TCEP, TDCPP, tris(butoxyethyl) phosphate (TBOEP), tris (2-ethylhexyl) phosphate (TEHP), triphenyl phosphate (TPH), cresyl diphenyl phosphate (CSDP), tricresyl phosphate (TCP), and 2-ethylhexyl diphenyl phosphate (EHDPP) were purchased from Tokyo Chemical Industry (Tokyo, Japan). Tripropyl phosphate (TPP), TCP, and triphenyl phosphate oxide (TPPO) were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). 6-Benzylbenzo[c][2,1]benzoxaphosphinine-6-oxide (6zIDOPO) was purchased from Sanko Co., Ltd. (Osaka, Japan). Naphthalen-2-yl diphenyl phosphate (NDPhP) was purchased from Biosynth AG (Staad, Switzerland). [5-ethyl-2-methyl-2-oxido-1,3,2-dioxaphosphorinan-5-yl] methyl methyl methylphosphonate (PMMP) and bis[5-ethyl-2-methyl-2-oxido-1,3,2-dioxaphosphanin-5-yl] methyl methylphosphonate (BPMMP) were purchased from Matrix Scientific (Columbia, SC, USA). 2,2-Bis(chloromethyl)-propene-1,3-diyltetraakis(2-chloroethyl) bisphosphate (V6) was purchased from Toronto Research Chemicals (North York, Canada). Isotope-labeled standards of TBP-d27, TCEP-d12, TPh-d15, TCP-d21, and TEHP-d51 were purchased from Hayashi Pure Chemical Industries Ltd. (Osaka, Japan).

Acetone, toluene, hexane, and ethyl acetate were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). Acetonitrile was purchased from Sigma-Aldrich (Tokyo, Japan). Milli-Q water (Merck KGaA, Germany) was used in all experiments.

**Sampling procedure**
Water samples (100 mL) were collected into a glass bottle from five water dispensers placed at commercial facilities and dwelling houses in Shizuoka, Japan, from July 2022 to April 2023. Before the sampling, the glass bottle was cleaned with acetone and hexane, and then baked at 500°C for 5 hours using an electric furnace.

**Extraction procedure**
To extract organophosphorus compounds from a water sample, liquid-liquid extraction was used. Briefly, 100 mL water sample, 100 mL ethyl acetate/hexane (1:1, v/v), 20 µL clean-up spikes (TPH-d15, TEHP-d51, TCP-d21, and TCEP-d12) was mixed in a separatory funnel, which was shaken for 30 minutes. The organic layer was transferred to a recovery flask and concentrated to approximately 1 mL using an evaporator (Nihon BUCHI K.K., Tokyo, Japan). Then, it was concentrated to 80 µL with a nitrogen purge, and 20 µL TBP-d27 was added as a syringe spike to make the final liquid volume of 100 µL.
**Analytical procedure**

The analysis of organophosphorus compounds in the drinking water collected from water dispensers was carried out by LC-MS/MS (Thermo Fisher Scientific Inc., Waltham, MA, USA) in atmospheric pressure chemical ionization (APCI) mode. A 2-µL aliquot of the extract was injected into the Accucore Vanquish C18 column (internal diameter: 2.1 mm, length: 100 mm, particle size: 1.5 µm) with water (Solvent A) and acetonitrile/methanol (1:4) (Solvent B) as the mobile phases at a flow rate of 0.3 mL min⁻¹. The column temperature was maintained at 50°C. The gradient program was as follows: isocratic at 5% solvent B for 0.5 min, isocratic at 100% solvent B for 6.5 min, isocratic at 100% solvent B for 8.0 min, isocratic at 5% solvent B for 0.1 min, and then isocratic at 5% solvent B for 1.9 min. The MS/MS was operated under selected reaction monitoring (SRM) mode. In this study, TMP, TEP, TPP, TBP, TEHP, TBOEP, TPhP, CsDPhP, EHDPhP, TCsP, TCEP, TCPP, TDCPP, TPhPO, BzIDOPO, NDPhP, PMMMP, BPMMP, and V6 were targeted.

**Quality Assurance/Quality Control**

The calibration curves for the organophosphorus compounds were linear over the concentration range of 1–1000 ng mL⁻¹ (1, 3, 10, 30, 100, 300, and 1000 ng mL⁻¹; \( R^2 > 0.99 \)). Good recoveries of the clean-up spikes (62–113%) were obtained from all the samples. The limit of quantification of the instrument was calculated as three times the standard deviation from five injections of organophosphorus compounds standards at low concentrations (at a signal-to-noise ratio of 3–10).

**Exposure and risk assessment**

The concentrations of organophosphorus compounds in drinking water were used to assess the oral ingestion rate associated with drinking water consumption. The following equation was used to estimate the daily intake rate, \( EDI \) (ng kg-bw⁻¹ day⁻¹).

\[
EDI = \frac{C \times DC}{BW}
\]

where \( C \) is the concentration of organophosphorus compounds in drinking water (ng L⁻¹), \( DC \) is the average intake rate of drinking water (L day⁻¹), and \( BW \) is body weight (kg-bw).

Non-cancer risks of organophosphorus compounds were evaluated using the hazard quotient, \( HQ \) (−), which was calculated by the following equation:

\[
HQ = \frac{EDI}{RfD}
\]

where \( RfD \) is the reference dose value of organophosphorus compound (ng kg-bw⁻¹ day⁻¹).

**Results and Discussion:**

**Concentrations of organophosphorus compounds in drinking water collected from water dispensers**

The concentrations of organophosphorus compounds in drinking water collected from 5 water dispensers placed at commercial facilities and dwelling houses in Shizuoka, Japan, are shown in Figure 1. Of the 19 organophosphorus compounds targeted in this study, nine types of them were detected with the detection frequency of more than 50%, which were TCEP (median concentration: 32 ng L⁻¹), TCPP (7.5 ng L⁻¹), TDCPP (7.5 ng L⁻¹), TPhP (1.0 ng L⁻¹), TEP (0.94 ng L⁻¹), TBP (0.64 ng L⁻¹), EHDPhP (0.49 ng L⁻¹), TEHP (0.30 ng L⁻¹), and CsDPhP (0.27 ng L⁻¹). TCEP, TCPP, and TDCPP were likely to be detected at higher concentrations compared with the others. In a previous paper that reported the occurrence of organophosphorus compounds in drinking water collected from water dispensers placed in China, TCPP was detected at the highest concentration (22.4 ng L⁻¹), followed by TCEP (1.31 ng L⁻¹). This result was in good agreement with those in this study. In other drinking waters reported in previous studies, TCPP, TCEP, and TDCPP were found in tap water (TCPP: 43 ng L⁻¹, TCEP: 48.5 ng L⁻¹, TDCPP: 5.8 ng L⁻¹), bottled water (TCPP: 0.6 ng L⁻¹, TCEP: 0.5 ng L⁻¹, TDCPP: 0.6 ng L⁻¹), barrel water (TCPP: 8 ng L⁻¹, TCEP: 6.9 ng L⁻¹, TDCPP: 0.5 ng L⁻¹), and well water (TCPP: 2.5 ng L⁻¹, TCEP: 0.5 ng L⁻¹, TDCPP: 0.1 ng L⁻¹). The concentrations of TCPP, TCEP, and TDCPP in drinking water collected from water dispensers placed in Japan were comparable to and/or higher than those in the conventional drinking waters.
Environmental and Human Exposure to Plasticizers and Other Consumer Product Chemicals

P-016  Organophosphorus Compounds in Drinking Water Collected from Water Dispensers in Japan

Figure 1: Concentrations of organophosphorus compounds in drinking water collected from water dispensers placed in Japan and other conventional drinking waters5-8. N.A.: Not Available

**Screening-level exposure and human health risk assessment**

The EDI values of organophosphorus compounds via consumption of drinking water from water dispensers in Japan are shown in Figure 2. The EDI value of TCEP was the highest (2.8 ng kg-bw−1 day−1) among organophosphorus compounds targeted in this study and followed by TCPP (1.2 ng kg-bw−1 day−1). Compared with the EDI values of organophosphorus compounds via dust ingestion and inhalation reported in previous papers9-11, which are recognized as significant exposure routes for organophosphorus compounds, those via consumption of drinking water from water dispensers could be comparable to and/or higher. These results suggest that the consumption of drinking water from water dispensers could be a major exposure pathway for organophosphorus compounds.

The estimated HQ values for organophosphorus compounds detected from drinking water collected from water dispensers in Japan using corresponding EDI values shown in Figure 1 ranged from 10−3 to 10−7. This result indicated that the human health risk via consumption of drinking water from water dispensers may not be concerned.

4. Conclusions:

The occurrence of organophosphorus compounds in drinking water from water dispensers placed in Japan was investigated. Chlorinated organophosphorus compounds, TCEP, TCPP, and TDCPP, were detected with high frequencies and concentrations. Screening-level exposure and human health risk assessment was carried out. The EDI values of TCEP, TCPP, and TDCPP were higher than the other organophosphorus compounds. According to the concentrations of organophosphorus compounds in drinking water from water dispensers obtained in this study, the human health risk via consumption of drinking water from water dispensers may not be concerned.

A more comprehensive campaign is required to obtain the whole picture of the occurrence of organophosphorus compounds in drinking water from water dispensers. In addition, a more refined exposure assessment is also desired.

Figure 2: Comparison of estimated daily intake rates of organophosphorus compounds via consumption of drinking water from water dispensers and other major exposure routes9-11. RfD: Reference Dose, N.A.: Not Available, N.D.: Not Detected.
5. Acknowledgments:
This research was supported by the Ministry of Economy, Trade and Industry, Japan, and a Grant-in-Aid for Scientific Research from the Ministry of Health, Labour and Welfare.

6. References:
1. Introduction:
Organophosphorus compounds are added as flame retardants and plasticizers into electronic devices (e.g., personal computers, television) and various indoor products, including plastic children’s toys and stuffed toys1,2. In the products, organophosphorus compounds are not chemically bound to the polymeric materials; therefore, they could migrate to the exposure media (e.g., indoor air, indoor dust) via volatilization, diffusion, and friction, resulting in human exposure3,4. Triphenyl phosphate (TPhP), one of the organophosphorus compounds, is of concern for endocrine disruption and metabolic dysfunction, reproductive and developmental toxicity, and cardiotoxicity5. Louis et al. 6 reported a significant association between urinary metabolite of TPhP levels and daytime asthma symptoms in a study of school-aged children.

The major exposure routes of organophosphorus compounds are inhalation via indoor air and dust ingestion via indoor dust; however, dermal exposure has great attention nowadays. TPhPs in nail polish have been experimentally confirmed to have dermal exposure via human skin7. Previous studies reported that dermal exposure to organophosphorus compounds through direct contact with products could be more important than inhalation and dust ingestion8-10.

In recent years, e-sports (electronic-sports) have become popular due to increased home time following the spread of COVID-19 infection and the cancellation of many traditional face-to-face sports competitions and events. In the 2020 Tokyo Olympics, the International Olympic Committee (IOC) and the International Sports Federations (IFSFs) organized an official e-sports competition. With the recent popularity of e-sports, there are more opportunities not only for young people to play games for long hours as a class or club activity in school but also for adults to play games professionally as professional gamers. Given that organophosphorus compounds could be included in plastic products, the increase in time to use home video game consoles may have a risk; however, the risk associated with the organophosphorus compounds in home video game consoles has not been evaluated. In addition, the occurrence and skin permeability of organophosphorus compounds in home video game consoles are limited.

In this study, the occurrence of organophosphorus compounds in home-use game console controllers, which are directly hand-contacted during their use, was investigated. The skin permeability of an organophosphorus compound in a controller was experimentally determined using artificial skin (EPISKIN). The screening-level of exposure assessment was also carried out.

2. Materials and Methods:

Chemicals and materials
Trimethyl phosphate (TMP), triethyl phosphate (TEP), tributyl phosphate (TBP), tris(2-chloroethyl) phosphate (TCEP), tris(1,3-dichloro-2-propyl) phosphate (TDCPP), tris(2-butoxyethyl) phosphate (TBOEP), tris(2-ethylhexyl) phosphate (TEHP), TPhP, cresyl diphenyl phosphate (CsDPHP), triresyl phosphate (TcSP), and 2-ethylhexyl diphenyl phosphate (EHDPHP) were purchased from Tokyo Chemical Industry (Tokyo, Japan). Tripropyl phosphate (TPP), tris(2-chloroisopropyl) phosphate (TCPB), and triphenyl phosphate oxide (TPhPO) were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). As for the (5-ethyl-2-methyl-2-oxido-1,3,2-dioxaphosphorinan-5-yl) methyl methyl methylphosphonate (PMMMP) and bis[(5-ethyl-2-methyl-2-oxido-1,3,2-dioxaphosphorinan-5-yl)methyl] methyl phosphonate (BPMMP) were purchased from Matrix Scientific (Columbia, SC, USA). Naphthalen-2-yi diphenyl phosphate (NDPHP), 6-benzylbenzo[c][2,1]benzoxaphosphinine 6-oxide (BzIDOPO), 2,2-bis(chloromethyl-propane-1,3-diyl)tetraakis(2-chloroethyl) bisphosphate (V6) were purchased from Biosynth AG (Staad, Switzerland), Sanko Co., Ltd. (Osaka, Japan), and Toronto Research Chemicals (Canada), respectively. Isotope-labeled internal standards of TBP-d27, TEHP-d51, TPhP-d15, TcSP-d21, and TcEP-d12 were purchased from Hayashi Pure Chemical Industries Ltd. (Osaka, Japan). Dichloromethane was purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan), and acetonitrile from Sigma-Aldrich Japan (Tokyo, Japan). EPISKIN (surface area = 1.07 cm2) was purchased from Nikoderm Research Inc. (Osaka, Japan). Dubecco’s Modified Eagle’s Medium-high glucose (DMEM) and acetonitrile were purchased from Sigma-Aldrich Tokyo (Japan). Bovine serum albumin (BSA), acetone, toluene, hexane, and ethyl acetate were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). Milli-Q water was used in all experiments. Brand-new 37 controllers for household videogame consoles, including 8 genuine and 29 third-party products, were purchased from the Japanese market in 2022.

Analytical Procedure for organophosphorus compounds in home video game controllers
Controller shavings (10 mg) were placed in a 10 mL test tube, adding 5 mL dichloromethane and sonicating for 30 minutes. The supernatant was diluted 10-fold, and the internal standard (100 ppb TBP-d27) was added with the final volume of 100 µL. The concentrations of 19 organophosphorus compounds (TMP, TEP, TBP, TCEP, TCPP, TDCPP, TBOEP, TEHP, TPhPO, TPhP,
CsDPhP, TCsP, EHDPhP, BzIDOPO, NDPhP, PMMMP, BPMMP, V6) were determined by using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) (Thermo Fisher Scientific Inc., MA, USA) in atmospheric pressure chemical ionization (APCI) mode. A 1.0 µL aliquot was injected into an Accucore Vanquish C18 column (length: 100 mm, internal diameter: 2.1 mm, particle size: 1.5 µm; Thermo Scientific, Inc.) with water (Solvent A) and 20% acetonitrile/methanol (1:4) (Solvent B) as the mobile phase at a flow rate of 300 mL min⁻¹. The column temperature was maintained at 50 °C. The gradient elution was programmed as follows: initial condition, 5% Solvent B; hold for 0.5 min; increase to 100% Solvent B over 7.0 min; hold for 8.0 min; return to initial condition over 0.1 min; hold for 1.9 min. Tandem mass spectrometry was conducted in selected reaction monitoring (SRM) mode.

Skin permeability test for the organophosphorus compound in home video game controllers using EPISKIN
The controller samples were cut into pieces of about 0.25 cm² using a nipper. DMEM culture with 5% BSA was used as a receptor fluid in the dermal permeability test. The receptor fluid was replaced during the trial at predetermined times (1, 2, 4, 8, 12, and 24 h). To determine the concentrations of organophosphorus compound transferred from the home video game controller to the receptor fluid, 2 mL receptor fluid was sampled, and 2 mL ethyl acetate/hexane (1:1, v/v) with internal standards (TPhP-d₁₅) was added into a test tube. It was vortexed for 1 min and centrifuged for 3 min (3000 rpm). The supernatant was aliquoted into a test tube and concentrated with a nitrogen purge to the final volume (100 µL) with the internal standard (TBP-d₂₇). The concentration of the organophosphorus compound was determined by using LC-MS/MS.

Estimation of potential dermal exposure rate
The skin permeation rate, J, (ng cm⁻² h⁻¹) was obtained from the experimental results of the skin permeability test. The following equation was used to estimate the potential daily dermal exposure rate of organophosphorus compounds associated with using home video game controllers, DED, (ng kg⁻¹ day⁻¹).

\[
DED = \frac{J \times S \times t}{BW}
\]

where S is the exposed skin area (cm²), t is the exposure time (h day⁻¹), and BW is the body weight (kg). The exposed skin area is the surface area of both palms, and the exposure time is the gameplay time per day.

A probabilistic dermal exposure assessment was performed using the Monte Carlo simulation with Oracle’s Crystal Ball software to account for the distribution of exposure parameters. The Monte Carlo simulation assumes an arbitrary distribution for the exposure parameters (e.g., normal, lognormal, triangular) and randomly extracts parameter values to estimate the potential dermal exposure rate. The number of trials was set to 100,000 for this simulation. In the Monte Carlo simulation, the surface area of both palms and body weight were assumed to be normally distributed, and the game playing time per day was assumed to be custom distributed according to literatures. In this study, four populations, such as students, students who belong to an e-sports club, adults, and professional gamers, were considered. These populations differ in body weight, the surface area of both palms and gaming time per day. The daily gaming hours were obtained from research firms’ questionnaires for students and adults. That for students who play e-sports as a club activity, was assumed to be 2 hours per day. As for professional gamers, the average gameplay time per day for practice was obtained by an internet survey.

3. Results and Discussion:
Occurrence of organophosphorus compounds in home video game controllers
Nineteen organophosphorus compounds in 37 home video game controllers were analyzed in this study (Figure 1). TPhP was detected in 5 genuine products and 22 third-party products, whose concentrations ranged from 1.0 to 530 µg g⁻¹. In previous studies, TPhP was detected in electronic devices such as televisions (600 µg g⁻¹) and laptop computers (500 µg g⁻¹)1,14. These results are consistent with those obtained in this study.
Environmental and Human Exposure to Plasticizers and Other Consumer Product Chemicals

P-017 Dermal Exposure to Organophosphorus Compounds in Home Video Game Controllers

Figure 1: Concentrations of organophosphorus compounds in home video game controllers purchased in the Japanese market. *ND: Not Detected.

Skin permeability test of the organophosphorus compound in the home video game controller using EPISKIN
The skin permeability test for TPhP in controller No. 37 (530 µg g⁻¹) was carried out. The skin permeation rate of TPhP was 0.42 ng cm⁻² h⁻¹.

Estimation of potential dermal exposure rate
The potential dermal exposure rates of TPhP via using home video game controllers for four populations were estimated. The experimental results are summarized in Figure 2. Daily dermal exposure rates of TPhP were estimated to be 0 (5%ile)-0 (50%ile)-7.7 (95%ile) ng kg⁻¹ day⁻¹ for students, 4.2-5.9-9.0 ng kg⁻¹ day⁻¹ for students who belong in e-sports club, 1.0-2.6-13 ng kg⁻¹ day⁻¹ for adults, and 9.0-22-45 ng kg⁻¹ day⁻¹ for professional gamers. The population of professional gamers showed the highest estimated potential daily dermal exposure rate among the four populations.

Compared with the exposure rates of major exposure routes reported in previous studies, the estimated potential dermal exposure rate of TPhP associated with using home video game controllers could be a significant exposure route for TPhP.

Figure 2: Comparison of exposure rates of TPhP via various exposure routes. *ND: Not Detected.

4. Conclusions:
The occurrence of organophosphorus compounds in home video game controllers was investigated. The controllers included TPhP with ND–530 µg g⁻¹. The skin permeability test of TPhP in the controller was carried out. Based on the test, the potential dermal exposure rate of TPhP associated with using home video game controllers was estimated. The estimated potential dermal exposure rate of TPhP could be higher than those via inhalation and dust ingestion, which are recognized as major exposure routes for TPhP. These results suggest that the dermal exposure to TPhP associated with using home video game controllers could be a major exposure pathway.
5. Acknowledgments:
This research was supported by the Ministry of Economy, Trade and Industry, Japan, and a Grant-in-Aid for Scientific Research from the Ministry of Health, Labour and Welfare, Japan.

6. References:
**Environmental and Human Exposure to Plasticizers and Other Consumer Product Chemicals**

**P-018 Occurrence of and human exposure to benzothiazoles and benzotriazoles in indoor dust in Suizhou and Beijing, China**

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**Introduction:** Previous experimental and epidemiological studies have shown that benzothiazoles (BTHs) and benzotriazoles (BTRs) may be associated with a variety of adverse health effects. As high-volume production chemicals, they have attracted public concern worldwide. Despite a few studies on these chemicals in different regions or countries, information that pertains to the occurrence and distribution of such chemicals in indoor microenvironment in China is still scarce. Therefore, investigations are needed to extend the knowledge about the occurrence and profiles of these chemicals in indoor dust, and exposure data of these pollutants from derived indoor dust are warranted.

**Materials and Methods:** Indoor dust samples were collected from two Chinese cities (Suizhou, a typical small city in central China; Beijing, the capital of China) from November 2018 to February 2019. A total of 79 indoor dust samples were collected from Suizhou (\(n = 70\)) and Beijing (\(n = 9\)). Dust samples were sieved with 0.18 mm stainless sieve, kept in aluminum foils, and stored at −20 °C until analysis. Approximately 100 mg of dust sample was weighed in a 15 mL glass tube, spiked with 50 µL internal standard (1000 ng/mL), and vortexed for 1 min. Target analytes were extracted with 5 mL of a mixed solution of hexane and dichloromethane (v:v = 1:1) under sonication (20 min each cycle, 3 cycles). The sample was centrifuged at 1811 g for 12 min. The supernatant was combined, filtered, concentrated under nitrogen to 1 mL, and then divided into two parts (0.5 mL each). One part was analyzed by ultra-high performance liquid chromatography–tandem mass spectrometry (UPLC-MS/MS). Before UPLC-MS/MS analysis, the solvent was exchanged to methanol. The other part was analyzed by gas chromatography–tandem mass spectrometry (GC-MS/MS). Prior to GC-MS/MS analysis, 20 µL MTBSTFA was added to each sample, and derivatization was conducted at 60 °C for 30 min.

**Results:** The concentrations, composition profiles, and human exposure of BTHs and BTRs in indoor dust were investigated. The concentrations and composition profiles of BTHs and BTRs in indoor dust from Suizhou and Beijing were investigated. The median concentrations of ∑6BTHs in indoor dust samples from Suizhou and Beijing were 133 and 439 ng/g dw, respectively, whereas the ∑5BTRs concentrations from Suizhou and Beijing were 28.4 and 40.1 ng/g dw, respectively. BTH, 2-OH-BTH, 1-H-BTR, and 5-Me-1-H-BTR were the predominant compounds in dust. Human exposure to such chemicals was further evaluated. The intake for population in Suizhou (0.163–0.939 ng/kg bw/day) and Beijing (0.0347–0.200 ng/kg bw/day), China was minor.

**Discussion and Conclusion:** Six BTHs and five BTRs were detected in indoor dust from Suizhou and Beijing. Levels of BTHs and BTRs were found higher in dust from Beijing than Suizhou. Human exposure to BTHs and BTRs from indoor dust was evaluated, and the intake for population in Suizhou and Beijing was minor. The obtained results in this study will provide baseline concentrations of such chemicals in indoor environment in China and be able to add insight into human exposure to BTHs and BTRs. It should be noted that this study on concentrations of BTHs and BTRs in Beijing is limited, due to the small sample size. Therefore, larger sample sizes need be examined in future study. In addition to the exposure from dust, other routes of exposure, including foodstuffs, and drinking water, should be investigated to evaluate the total burden of BTHs and BTRs in human body.

**Acknowledgments:** This work was financially supported by the National Key Research and Development Program of China (2020YFA0907500), the National Natural Science Foundation of China (22193051 and 22225605), and the K.C. Wong Education Foundation of China (GJTD-2020-03).
Distributions and potential sources of traditional and emerging polycyclic aromatic hydrocarbons in sediments of the Pohang Harbor, South Korea

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Introduction: Polycyclic aromatic hydrocarbons (PAHs) are mainly produced through incomplete combustion of materials, such as during coke manufacturing or steel sintering processes (Menzie et al., 1992; Liberti et al., 2006). Pohang, located in the southeastern part of South Korea, is home to highly developed iron and steel industries and an active harbor for maritime logistics. Pohang Harbor is known to be the most severely polluted by PAHs in South Korea due to the influence of the surrounding steel industry and ports. It is necessary to study the identification of PAHs sources and the contribution of each source for the protection of marine environments. The objectives of this study are to investigate the distribution and potential sources of PAHs in the sediments of Pohang Harbor. In addition, compound-specific isotope analysis (CSIA) of PAHs in sediments was applied to identify the sources.

Materials and Methods: Twenty-six surface sediments were collected from Pohang Harbor in June 2022. All sediment samples were freeze-dried and sieved (2-mm mesh), and then homogenized. The sediments were extracted using an accelerated solvent extractor and then purified through 8.0 g activated silica gel column. Fifteen traditional PAHs (t-PAHs) and thirteen emerging PAHs (e-PAHs) were quantified using a gas chromatography-mass selective detector. To identify the potential sources of t-PAHs and e-PAHs in sediments, diagnostic ratios and positive matrix factorization (PMF) models were used. For the CSIA of PAHs, HPTLC was used to remove the unresolved complex mixture from the organic extract. Carbon stable isotope ratios of 9 PAHs were measured using gas chromatography-isotope ratio mass spectrometry.

Results: The concentrations of t-PAHs and e-PAHs in the sediments of Pohang Harbor were 390–8200 ng g⁻¹ dry weight (dw) (mean: 1900 ng g⁻¹ dw) and 65–1200 ng g⁻¹ dw (mean: 350 ng g⁻¹ dw), respectively. Distribution patterns of t-PAHs and e-PAHs showed similar trends at all sites. The concentrations of PAHs in the sediments were relatively high at sites close to the steel industry. Fluoranthene (Fl) accounted for a large proportion of the PAHs composition in the sediments. At all sites, four- to six-ring PAHs were the predominant PAHs, accounting for more than 80% of the total PAHs composition. Diagnostic ratios of PAHs indicated that coal combustion and petroleum combustion are the main sources of PAHs in Pohang Harbor. The PMF model identified three factors, such as coal combustion (Factor 1), petroleum combustion (Factor 2), and fossil fuel combustion (Factor 3). Fossil fuel combustion (53%) was identified as the major source of PAHs in Pohang Harbor. The PMF model identified three factors, such as coal combustion (Factor 1), petroleum combustion (Factor 2), and fossil fuel combustion (Factor 3). Fossil fuel combustion (53%) was identified as the major source of PAHs in Pohang Harbor. HPTLC was used to remove the unresolved complex mixture from the organic extract. Carbon stable isotope ratios of 9 PAHs were measured using gas chromatography-isotope ratio mass spectrometry.

Discussion and Conclusion: In this study, the distributions and potential sources of PAHs in the sediments of Pohang Harbor were determined. Concentrations of sedimentary PAHs were shown to be relatively high at sites close to the steel industry. It is known that 4–6 rings PAHs are mainly generated through the combustion of fossil fuels. At all sites, high molecular weight PAHs account for more than 80% of the total concentrations, which seemed to be affected by the combustion of fossil fuels in the steel industry. Typically, Fl is considered to be generated through coal combustion. Coal combustion in the steel industry causes the production of Fl, which appears to accumulate in nearby harbor sediments. Source identification of PAHs based on molecular composition may not be conservative due to redistribution between environmental media; thus, it is necessary to apply more conservative and multiple source indicators, such as compound-specific carbon and hydrogen stable isotope ratios.

Acknowledgment: This research was supported by the "Development of Source Identification and Apportionment Methods for Toxic Substances in Marine Environments (20220534)" program of the Korea Institute of Marine Science & Technology Promotion (KIMST) funded by the Ministry of Oceans and Fisheries.

References:
Reliable quantitation of per/polyfluoroalkyl substances (PFAS) in water samples has been a significant challenge for environmental labs in recent years. The most commonly quantified legacy and emerging PFAS belong to a number of different chemical classes and exhibit varying degrees of hydrophobicity, which makes it more challenging to develop simple robust methods for routine quantitation in a range of water matrices. Offline or online sample enrichment can be employed, but a direct injection method leveraging the sensitivity of high-end LC-triple quadrupole (LC-TQ) systems would be preferable for many routine testing labs. The aim of this work was to develop and validate a LC/MS-MS method with direct injection, targeting a range of 47 PFAS of interest to water testing labs globally and especially to labs in the United Kingdom.

Calibration standards, spiked with known concentrations of 47 PFAS, were prepared by serial dilution. 23 isotopically labelled internal standards were added to ensure accurate quantitation. Calibration standards were prepared using ultrapure water, and matrix spiked samples were prepared at three different levels (blank, low and high concentration) in four different matrices, including drinking water, groundwater and surface water matrices. PFAS-free materials were used throughout sample preparation. The samples were analysed using a high sensitivity LC-TQ system, with a chromatographic run time of 18 min and a C18 column used for the chromatographic separation. The method was validated through the analysis of 12 separate batches, each including blanks, calibration standards, spiked samples, and matrix spiked samples.

Limits of detection (LODs) of < 10 ng/L were achieved for all analytes, and limits of quantitation (LOQs) < 30 ng/L were achieved for all analytes. All analytes met the following requirements of the validation process: precision < 12.5 %, uncertainty of measurement (UoM) < 60 %, and bias < 25 %. The method was accredited by United Kingdom Accreditation Service (UKAS) to meet the requirements of ISO 17025.

This method addressed the challenges associated with quantifying PFAS of varied hydrophobicities via a direct injection method requiring only a single injection per sample. While there are alternative approaches to these analytical challenges, such as offline or online sample enrichment, these are more complicated, more time-consuming, and can introduce additional sources of PFAS contamination. As such, a direct injection method such as that reported herein has the potential to streamline PFAS quantitation for many water testing labs. The validation in accordance with ISO 17025 demonstrates that the method is fit-for-purpose for water testing labs whose target lists for PFAS are covered by the 47 analytes analysed in this work.

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Polychlorinated dibenzop-dioxins (PCDD) and polychlorinated dibenzop-furans (PCDF) are widely distributed in the environment. The sources of PCDD and PCDF are widely described; one of these sources is the wildfires. Active air samplers were installed in the city of Concepcion during the occurrence of wildfires. Samples were then carried to the laboratory and stored in cold and dark. Fractions, gas and particulated where then extracted with a mixture of DCM: Hexane 1:1 in a PLE-FMS System and purified with a Power Prep system using Silica, Alumina and Carbon columns. The analysis was performed using HRGC-HRMS Thermo Scientific DFS mass spectrometer. Extraction, cleanup and analysis were based on the method EPA-23. Concentrations from gas and particulate fraction where then used to calculated the total concentration in the sample, the results were expressed as ng*m³ of air sampled. The results showed the presence of PeCDD, HxCDD, HpCDD, HpCDF, OCDD and OCDF in all sampled coming from the wildfires.

Acknowledgements:
Authors acknowledge to the administration of the "Laboratorio de Oceanografía Química" of Universidad de Concepcion, for their financial support to carry out this research. The staffs of the laboratory are thanked to their contribution in the acquisition of chemicals supplies that allowed extraction, analysis and sampling.
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Introduction: The laboratory assessment on Persistent Organic Pollutants (POPs)[1] has distinctly increased in the last decades. InterCinD is a division of LabService Analytica[2] and has being involved for more than 20 years in proficiency testing (PT) of POPs as PT provider accredited ISO/IEC 17043:2010 (accreditation nr PTP0007, given by the Italian authority for accreditation, i.e. ACCREDIA). The main distinctive characteristics of InterCinD relies on:

1. The use of natural contaminated matrixes, which are properly treated, prepared according to the ISO Guide 34:2009, and preliminary assessed for their homogeneity and stability before being sent to the laboratories;
2. Samples are delivered to laboratories which provide results of three replicates which permit to evaluate the performance of laboratories in terms of precision and accuracy;
3. Concentrations of the target pollutants are unknown and the assigned value for each chemical species is determined not from expert labs but from participants, after a statistical treatment of the dataset (implying the determination with no parametric methods and removal of extremes) and a rigorous evaluation in compliance with the ISO 13528/2022 and IUPAC Guidelines and the final determination of the "consensus" value and its uncertainty.

PFAS – e.g. PFOS, PFOA and PFNA - have drawn attention to environmental and health control agencies due to their persistence, hypothetical toxicity and wide environmental diffusion. In 2009 the PFAS were recognized as "persistent organic pollutants" under the Stockholm Convention[3]. Because of this growing interest, since 2020 InterCinD started as an Italian pioneer to propose a specific PT focused on this class of compounds.

Materials and Methods: Here we present the comparison of the InterCinD methodological approach and the three main methods suggested in the ISO 13528/2022 – i.e. Algorithm A; Q/Hampel method and MADe - by evaluating the results of three distinctive PTs for PFAS in water samples.

Results: The InterCinD performs well in terms of breakdown point, efficiency and resistance to minor modes independently from considering different data distribution.

Discussion and Conclusion: The results demonstrate that there is no statistical method that is perfect for all situations. The sample mean and standard deviation are optimal with a normal distribution but break down in case of outliers. Simple robust methods such as median, Algorithm A or nIQR perform comparatively worse for normally distributed data but can be effective when outliers are present or the data set is small. Results depend heavily on the underlying distribution of results for a population of competent participants, or for population from participants incompetent or that did not follow instructions or the measurement method. The resultant contaminating data can appear as outliers or extremes, results with larger variance, or results with a different mean (e.g. bimodal).

The InterCinD has the advantage to use conventional parameters to estimate the population, after the identification of extremes values, and to identify alternative approach (mean and standard deviation or median and nIQR) on the basis of data distribution by applying simple statistical consequential criteria to explore the dataset.

References:
2. InterCinD [WWW Document] available at: https://www.intercind.eu/it/
Introduction: The Black Sea coast of the southern region of Ukraine is a primary transport and industrial hub, and therefore the ecosphere of this region is undergoing a robust anthropogenic impact. At the same time, this region is actively used as a recreational area. Thus, monitoring the content of hazard and toxic pollutants are essential for ecological and human health risk assessment and for risk management activities. Here we present the results of the determination ones of polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) in the samples of bottom sediments, algae, tissues of fishes and marine mammals within the framework of monitoring of the ecological state of the Black Sea region of Ukraine.

Materials and Methods: The samples of the tissues of a fish and dolphin were cut into small pieces and ground with anhydrous sodium sulfate as a desiccant. The algae samples were air-dried at room temperature for 48h and ground. After being air-dried at ambient temperature, sediment samples were thoroughly mixed and ground with anhydrous sodium sulfate. Then, the 13C-labelled internal standards were added, and the samples were extracted with the mixture of hexane and dichloromethane in the Randall extractor for 8-12 hours in an automatic extractor at 4-6 cycles/h.

Concentrated extracts were purified by the automatic sample preparation system using the kit of cartridges with acidic silica gel, alumina and activated carbon. PCDDs and PCDFs (17 contaminants), dioxin-like PCBs (12 analytes) and non-dioxin-like PCBs (6 compounds) were analyzed with isotope dilution technique using gas chromatography coupled to high-resolution mass spectrometry (GC-HRMS). The quality control samples (QQS) were analyzed together with the samples to verify the accuracy and precision of the measurements.

Results: The tested sediment samples are contaminated with dioxins to a greater extent than with furans. OCDD (1.3-59.3 pg/g) and 1234678-HpCDD (0.28-4.53 pg/g) predominate among dioxins. Among dioxin-like PCBs, most of all were found PCB105 (258-451 pg/g), PCB118 (541-1091 pg/g), PCB114 (15.3-29.7 pg/g). The sum of non-dioxin-like PCBs was in the range of 1.63-5.78 ng/g.

Concentrated extracts of the tested sediment samples were contaminated with dioxins and furans. OCDD (1.3-59.3 pg/g) and 1234678-HpCDD (0.28-4.53 pg/g) predominate among dioxins. Among dioxin-like PCBs, most of all were found PCB105 (258-451 pg/g), PCB118 (541-1091 pg/g), PCB114 (15.3-29.7 pg/g). The sum of non-dioxin-like PCBs was in the range of 1.63-5.78 ng/g.

Among the fish samples, there were samples in which the concentration of PCDDs and PCDFs was <LOQs and only dioxin-like PCBs were present in low concentrations: PCB77 0.35-0.53 pg/g, PCB105 5.6-7.1 pg/g, PCB118 14.3-19.8 pg/g. The samples of the other kinds of fishes were contaminated to a greater extent: 2378-TCDD n.d.-0.061 pg/g, 12378-PeCDD 0.042-0.100 pg/g, 2378-TCDF 0.195-0.734 pg/g, 12378-PeCDF 0.045-0.084 pg/g, 23478-PeCDF 0.050-0.246 pg/g, 1234678-HxCDF n.d.-0.073 pg/g. These samples also contain almost all the tested dioxin-like PCBs, with a predominance of PCB105, PCB118, PCB156, PCB167: 62.3-155.4, 211.5-557.6, 15.1-58.4, 11.4-46.2 pg/g, respectively. The sum of non-dioxin-like PCBs was in the range of 1.06-2.97 ng/g. The samples of dolphin tissues showed the presence of 0.099 pg/g of 12378-PeCDD, 0.116 pg/g of 23478-PeCDF, 0.0146 pg/g of 234678-HxCDF, the rest of the PCDDs and PCDFs were <LOQs. Among dioxin-like PCBs, most of all were found PCB105 (1716.2 pg/g), PCB118 (8880.5 pg/g), PCB156 (193.7 pg/g), PCB167 (329.1 pg/g), PCB114 (165.5 pg/g). The sum of non-dioxin-like PCBs was 65.7 ng/g with the highest content of PCB153 (29.7 ng/g), PCB138 (18.4 ng/g), PCB180 (9.3 ng/g).

Discussion and Conclusion: Dioxin-like contaminants are up taken from bottom sediment by benthic organisms, which become food for individuals at the higher levels of the aquatic food chain. Therefore, sediments are considered the main source of dioxins for aquatic organisms. The results have shown that bottom sediment and algae could be indicators of general pollution levels and local emissions, respectively. The profiles of contents of PCDDs, PCDFs and PCBs are characteristic of sources related to industrial waste incineration, and the operation of petrol and diesel engines. PCB118 and PCB105 congeners, which are predominant in the sediments, algae, fish and mammal muscles, may also be derived from emissions from metallurgical processes. High levels of OCDD may also be due to environmental pollution by chlorophenols and dioxin-contaminated pesticides. The results of this study proved the significant influence of environmental contamination on the biota of the Black Sea region of Ukraine.

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European POPs Monitoring

P-024 Monitoring of HCH, PeCB and HCB in air from a former HCH production site in Spain

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1. Introduction:
The landfilling and dumping of persistent organic pollutants (POPs) and other persistent hazardous chemicals, such as hexachlorocyclohexane (HCH) isomers, pentachlorobenzene (PeCB) and hexachlorobenzene (HCB) can have significant adverse environmental consequences and cause contamination in soil, water, and atmosphere systems1.2. Among HCH isomers, only γ-HCH has a specific pesticide activity and its purification resulted in the production of other waste residues. In Spain, the manufacture of lindane has been associated with four production sites that generated nearly 200,000 t of HCH wastes3. Approximately 65% of these wastes were generated by the INQUINOSA Factory located in Sabiñánigo (Aragón, Spain), from 1975 to 1992, and were mainly dumped at Bailín and Sardas landfills. Remediation and management of dumpsites is a worldwide problem that must be addressed to protect human health and the environment. Since 2007, a considerable investment has been done in activities focused on the remediation and containment at the HCH production site as well as to secure landfills, framed in a project plan approved by the Government of Aragón. To protect and assess the local environment, the concentrations of HCH isomers in air were periodically monitored in the Bailín and Sardas landfills and surroundings since the Bailín dismantling works to the present. This work assesses the monitoring of HCH, PeCB and HCB in the air of the influenced environment of Sabiñánigo.

2. Materials and Methods:
The present study includes 657 air samples collected in 74 consecutive sampling campaigns (SC) conducted from summer 2014 to autumn 2021. Ten sampling points were selected at the Bailín and Sardas influence area - Bailín area: P1-P4 (near the landfill) and P5 (in the town of Sabiñánigo); Sardas area: P7 and P8 (possible sources of pollution) and P6, P9 and P10 (residential sites). While HCH isomers were monitored in all locations (from 1st campaign at Bailín area and 17th at Sardas area), chlorobenzenes (CBs) were determined in P7 and P10 from the 24th campaign. At each sampling point, one passive air sampler with a polyurethane foam (PUF) disk was deployed for a month. PUF disks were precleaned by Soxhlet extraction with acetone and diethyl ether for 24 h, then wrapped in aluminum foil and stored in polyethylene bags at -20 °C until deployment. Compound-specific sample air volumes were calculated following the Tom Harner Template5.

Samples spiked with 13C-labeled surrogate standards (13C6-α-, β-, γ- and δ-HCH, 13C6-PeCB and 13C6-HCB) were Soxhlet extracted in toluene for 24 h. The extracts were solvent exchanged into hexane and purified by Florisil column. The elution was carried out with n-hexane and n-hexane:chloromethane (50:50, v/v). The final extracts were concentrated under a nitrogen stream, redissolved in nonane and spiked with the 13C injection standards solutions (13C12-PCB 15 and 13C12-PCB 70) prior to instrumental analysis. Target analytes (α-, β-, γ- and δ- HCH isomers, PeCB and HCB) were analyzed on a Varian CP-3800 gas chromatograph connected to a 320 MS-TQ mass spectrometer. Quantification was carried out using isotopic dilution method. Isomer concentrations were higher than LODs in all cases. Instrumental blanks (nonane) were run before each sample injection to check instrumental contamination. Field blanks were taken at each sampling campaign and analyzed as samples. Data were blank corrected.

3. Results and Discussion:
The concentrations of HCH in the different areas considered were shown in Figures 1 and 2. Important differences were found between sampling locations and sampling campaigns.

HCH concentration in Bailín area
HCH concentrations obtained during the first two sampling campaigns -α-HCH (224 ng/m3; median), β-HCH (174 ng/m3), γ-HCH (49 ng/m3), δ-HCH (56 ng/m3) and ε-HCH (28 ng/m3)-, presented statistically higher concentrations (Kruskal-Wallis p < 0.01) for all isomers compared to those obtained afterwards -α-HCH (3.9 ng/m3; median), β-HCH (0.6 ng/m3), γ-HCH (1.6 ng/m3), δ-HCH (1.7 ng/m3) and ε-HCH (0.2 ng/m3). Levels detected were in accordance to those associated to historical production sites2. Considering that the first two sampling campaigns covered the dismantling of the old landfill and the subsequent sealing of the new cell, results indicate that the works performed during the dismantling were a source of HCH contamination. After those, a clear decrease in HCH levels was observed in all sampling points. Nevertheless, some interesting differences between sampling sites were observed: P1 and P2 continued to present having statistically higher values than P3 and P4 and these were higher than those derived from P5. This geographical distribution highlighted that old landfill represents a HCH source even after dismantling work has been completed. Besides, statistically positive correlations were found among HCH isomers in all sampling points, showing a major common source.

Once dismantling works finished, an equilibrium state was reached (from SC5 to SC74) and a similar isomer profile was obtained at P2-P5: α-HCH (45± 5%, mean± SD) followed by γ-HCH (24 ± 5%) and δ-HCH (22 ± 56), and to a lesser extent by β-HCH (7 ± 2%, mean ± SD) and ε-HCH (2 ±1%). A different isomer profile was observed at P1, being γ-HCH the predominant isomer (41± 8%) instead of α-HCH, reflecting the possible influence of DNAPL (Dense Non-Aqueous Phase Liquid) caption cell.
**HCH concentration in Sardas area**

The sum of α-, β-, γ-, δ- and ε-HCH (ΣHCH) concentration in the area evaluated ranged between 0.03 and 64.8 ng/m³. ΣHCH air concentrations obtained at Sardas landfill (P7; 5.11 ng/m³, median) and INQUINOSA Factory (P8; 3.36 ng/m³) showed statistically higher values than the other locations (0.48, 0.51 and 0.23 ng/m³ for P6, P9 and P10, respectively). This result clearly highlights these facilities as currently HCH air pollution sources. Samples obtained from P6, P7, P8 and P10 showed a similar isomer profile with a higher contribution of α-HCH (49 ± 9%, 37 ± 9%, 46 ± 10% and 44 ± 8%; mean ± SD at P6, P7, P8 and P10, respectively) followed by γ-HCH (28 ± 7%, 35 ± 7%, 29 ± 6% and 31 ± 7%), δ-HCH (15 ± 5%, 19 ± 6%, 17 ± 7% and 16 ± 5%), β-HCH (7 ± 4%, 5 ± 3%, 6 ± 3% and 7 ± 4%) and ε-HCH (2 ± 1%, 3 ± 2%, 2 ± 2% and 2 ± 1%). However, in P9 case, the main isomer was γ-HCH (52 ± 11%) followed by α-HCH (29 ± 7%), this suggests the presence of a lindane source hitherto unknown with significant influence at this site.

**PeCB y HCB concentration in Sardas area**

CB air concentrations ranged from 0.6 to 318 pg/m³ for PeCB and from 1.0 to 98 pg/m³ for HCB. Positive correlations were found between PeCB and HCB at the two sampling points evaluated (P7: r > 0.394, P10: r > 0.605; p < 0.01), suggesting a major common origin for both chlorobenzenes. PeCB concentrations were statistically higher in P7 (83 pg/m³, median) than P10 (8.4 pg/m³). Levels detected were comparable to values observed in other studies.

**4. Conclusions:**

To date, passive air samplers have been mainly used to monitor background concentrations at remote locations. However, in light of results obtained here they also revealed as a reliable method to monitor polluted environments.

Results highlight that despite of remediation and containment measures implemented in Sabiñánigo, further pollution control activities should be carried out to protect the environmental compartments and the human health.

Besides, this research reveals the necessity of suitable air monitoring plans in those mega-sites where obsolete pesticides were improperly dumped.

**5. Acknowledgments:**

The present study has been supported by the Government of Aragón, through the public company SARGA under the contracts no, 5506079-17, 5506079-29, 5507001-18 and 5500011-05.

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Figure 1. Concentration (ng/m³) of HCH in air at different sampling points from Bailín and Sardas area; outliers (circles) and extreme (asterisks) values were labeled with sampling campaign code.
Figure 2. Concentration (ng/m³) of HCH in air and temperature influence in the different sampling campaigns (from SC3 to SC74).
Introduction: The overuse of soil by intensive agricultural practices, causes its erosion, desertification and deterioration, and makes the soil inaccessible to future generations as a resource. One possible way to reverse these negative effects is by increasing the organic carbon and nitrogen stocks of the soil with organic amendments such as sewage sludge; this has been recommended as one possible method of sludge utilization by the European Directive on Sewage Sludge (86/278/EEC). However, as the end product of wastewater purification processes, sewage sludge contains large amounts of toxic substances (xenobiotics), including legally controlled ones and those that are not subjected to any regulation (emerging contaminants). Hence in recent years, the use of sewage sludge has become a major safety concern for its potential risk to the environment as well as human health. Considering the above, the aim of our study was to test the overall quality of sewage sludge in terms of its safe utilization in agriculture.

Materials and Methods: The sewage sludge were collected 5 times throughout the year 2021-2022 (i.e. every 2 months) from four Wastewater Treatment Plants (WTPs) differing in size (small – Class I, medium – Class II, medium-large -Class III, large – Class IV) and purification technology. The collected sewage sludge were tested in term of their chemical composition (content of heavy metals, PCDDs/Fs, PCBs, and antibiotic residues), ecotoxicity (Microtox, Ostracodtoxkit), and potential health (hazard quotients – HQ) and environmental risk (risk quotients RQ).

Results: The obtained results indicate a significant diversification of the level of pollutants in individual WTPs. In term of heavy metals, the highest average concentration was noted for Zn (459 mg/kg), following by Ni (399 mg/kg), Cu (100 mg/kg), Cr (25 mg/kg), Pb (16.8 mg/kg), and Cd (6.34 mg/kg). The WTP with the most elevated concentration of heavy metals was Class III WTP, while Class I WTP demonstrated the lowest content of heavy metals. PCDDs/Fs and WHO-PCBs and PCBi contents were the highest in Class III WTP (14 ng TEQ/g, 1.3 ng TEQ/g and 15 ng TEQ/g, respectively), following by Class IV WTP (4.5 ng TEQ/g, 0.82 ng TEQ/g and 18 ng TEQ/g, respectively), Class II WTP (5.7, 0.50 and 4.1 ngTEQ/g, respectively), and Class I WTP (2.3, 0.46 and 4.8 ngTEQ/g, respectively). All tested sewage sludge demonstrated also elevated contents of antibiotics, with the highest average concentrations noted for tetracycline (337 ng/g), following by ofloxacin (117 ng/g), ciprofloxacin (88 ng/g) norfloxacin (78 ng/g), trimethoprim (10 ng/g), clindamycin (3.3 ng/g) and thiamphenicol (0.48 ng/g). Among all four WTPs, tetracycline, trimethoprim and clindamycin were noted in the highest concentrations in sewage sludge coming from Class I WTP; while ciprofloxacin, norfloxacin, thiamphenicol and ofloxacin showed the highest average concentrations in class II WTP. The ecotoxicity test confirmed high toxicity (80-100% PE%) of the studied sewage sludge for both test organisms i.e. *Vibrio fisheri* (Microtox) and *Heterocypris incongruens* (Ostracodtoxkit). Additionally, risk assessment proved a potential health hazard of the studied sewage, which was confirmed by elevated Hqs: 0.022 - 1.33 for heavy metals, 0.023 - 0.14 for PCDDs/Fs, 0.009 - 0.01 for iPCBs and 0.005 -1.57 for antibiotics. The ecological risk assessment of agriculture-used sewage sludge revealed high Rqs (<1) for antibiotics mostly from small and medium WTPs. The highest Rq=729 was obtained for tetracycline in sewage sludge from small WTP. None of analyzed heavy metals exceeded Rq of 0.01.

Discussion and Conclusion: The presented study, through a multidimensional approach to the problem of safe utilization of sewage sludge, indicates a necessity to evaluate a wide range of factors (chemical, ecotoxicological and risk assessment) in assessing the overall quality of sludge for the purpose of its application in agriculture. Due to the lack of legal regulations regarding the permissible concentrations of emerging contaminants (e.g. antibiotics) in sewage sludge, the issue of their safe disposal in agriculture remains a pressing problem, which should be addressed by both scientists and legal authorities.

Acknowledgments: Research funded by the National Science Center, Poland, under research project No. 2020/39/B/NZ9/01772 entitled Microbial community, antibiotic resistance and physicochemical changes in soil amended with municipal sewage sludge (contract nr UMO-2020/39/B/NZ9/01772)
Fate and Transport

P-026  Source Apportionment, Hydrodynamic Influence, and Environmental Stress of Pharmaceuticals in the Pearl River Estuary in South China

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Introduction: In recent decades, pharmaceuticals in the environment have been regarded as emerging chemicals of concern (ECCs). In estuaries, tidal currents can significantly influence water quality parameters. However, existing field data is insufficient for an assessment of the impact of tidal events and the subsequent changes in water quality parameters on the geochemical processes and transport of pollutants, especially ECCs like pharmaceuticals. Under the policy intervention on pharmaceutical prescription and continuous increase of pharmaceutical expenditure in the Pearl River Estuary (PRE), South China, it is of concern whether the updated presence of pharmaceuticals can pose significant risks to the marine environment of the PRE. Thus, the present study comprehensively investigated the occurrence of antibiotics and psychiatric pharmaceuticals in the PRE, examined the influence of tidal events at the eight major Pearl River outlets and hydrodynamic conditions on the fates of pharmaceuticals, and estimated the environmental capacities and stress of pharmaceuticals at the eight outlets by modeling.

Materials and Methods: This study investigated the presence of 40 pharmaceuticals in water and sediment of the Pearl River Estuary (PRE) in the wet season of 2020. The analysis of the target analytes used ultra-performance liquid chromatography-tandem mass spectrometry. Water quality parameters (i.e., total nitrogen, total phosphorus, salinity, dissolved oxygen) were measured. Environmental capacity estimation of antibiotics was performed using a semi-implicit Eulerian-Lagrangian finite-element (SELFE) numerical model. Source apportionment was performed using Positive Matrix Factorization, following standard procedures.

Results: Among psychiatric drugs, only diazepam was found in water samples, while six of them was detected in the sediment. The antibiotics levels ranged from 6.18 ng/L to 35.9 ng/L and 2.63 ng/g dry weight to 140 ng/g dry weight in water and sediment samples, respectively. Low ecological risks to the aquatic organisms and of causing antimicrobial resistance were identified. Likewise, hydrological modeling results revealed insignificant risks: erythromycin-H2O and sulfamethoxazole discharged through the outlets constituted 30.8% and 6.74% of their environmental capacity. Source apportionment revealed that pharmaceutical discharges through the Humen and Yamen outlets were predominantly of animal origin.

Discussion and Conclusion: The levels of antibiotics found in the PRE in the present study were historically low compared to those in other coastal areas from studies conducted in the same region, nationwide, and on a global scale. This could be ascribed to stricter regulations on antibiotic prescription and the ban on the use of some antibiotics (e.g., fluoroquinolones) for veterinary use.2 COVID-19 pandemic could be another possible reason as reduced consumption of antibiotics has been observed in China. In the outlet sediment, fluoroquinolones and tetracyclines were found well settling, which was related to either their higher hydrophobicity or more binding sites with the metal ions, compared with other classes of antibiotics (e.g., sulfonamides).3, 4 Sulfonamides, including sulfamethazine, sulfadiazine, and doxycycline, were positively correlated with flow rate (Pearson, r < 0.05), which could be explained by the release from disturbed sediment under stronger tidal wash-out conditions. After entering the marine waters, pharmaceuticals tended to deposit at the PRE mouth by the influence of plume bulge and onshore invasion of deep shelf waters. Overall, our findings provide strategic insights on environmental regulations to further minimize the environmental stress of pharmaceuticals in the PRE.

Acknowledgments: The present work was supported by the Innovation Group Project of Southern Marine Science and Engineering Guangdong Laboratory (Zhuhai) (No. 311020004) and the Science, Technology, and Innovation Commission of Shenzhen Municipality (No. JCYJ20190812155805559).

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Fate, Detection and Analysis of Chlorinated Paraffins

P-027 Occurrence of short- and medium-chain chlorinated paraffins in edible insects from Europe and Asia

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Introduction: Edible insects are considered a suitable alternative to the continuous request for animal proteins caused by the growing world population's increasing demands (Lee et al., 2021). Despite their proven beneficial traits, which include elevated nutritional value and environmental sustainability, the chemical safety of edible insects remains a major factor determining their inclusion in the human diet (Poma et al., 2019). As with other foodstuffs, however, insects may accumulate potentially harmful contaminants, including chlorinated paraffins (CPs). In this study, we investigated the presence and patterns of short- (SCCPs) and medium-chain (MCCPs) CPs in edible insects purchased from different European and Asian countries. The potential for exposure to CPs associated with insect consumption (i.e. estimated daily intake, EDI) among adult populations was also evaluated.

Materials and Methods: A total of 36 edible insect samples (n=24 from Asia: South Korea and Japan; n=12 from Europe: Austria, Belgium, the Netherlands, and United Kingdom) authorized for human consumption were purchased and analyzed for SCCPs and MCCPs via gas chromatography and mass spectrometry (GC-ECNI/MS) according to the protocol described by McGrath et al., 2021.

Results: SCCPs were detected in 83% of all edible insect samples with an overall median ∑SCCP concentration of 8.7 ng/g dry weight (dw) and a range of <2.0 to 410 ng/g dw, while MCCPs were present in 92% of samples with a median ∑MCCP concentration of 51 ng/g dw and a range of <6.0 to 380 ng/g dw. Concentrations of ∑MCCPs were greater than those of ∑SCCPs in 81% of samples with an average SCCP/MCCP ratio of 0.26. The 50th percentile deterministic EDIs were 0.19 and 0.092 ng/kg bw/d for Asian and European adult populations, respectively, for ∑SCCPs, and were 1.5 and 0.27 ng/kg bw/d for Asian and European adults, respectively, for ∑MCCPs.

Discussion and Conclusion: Median ∑SCCP and ∑MCCP levels in edible insects purchased in Asia were approximately two- and four-times higher, respectively, than those from Europe, while the difference was statistically significant for ∑MCCPs (p < 0.001). Differences in homologue patterns were also observed between Asian and European samples to suggest diverse sources of CP contamination to insects which may include environmental accumulation, industrial processing equipment and food additives. Estimated daily intake of SCCPs and MCCPs via consumption of edible insects were generally lower than intake from animal products in other studies and suggested that adverse health outcomes were very unlikely, but that continued monitoring of insect farming and processing practices are warranted.

References:
Fate, Detection and Analysis of Chlorinated Paraffins

P-028 Chlorinated paraffins in seaweed chips – First insights using High-Resolution Mass Spectrometry

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Introduction: Chlorinated paraffins (CPs) are complex mixtures of polychlorinated n-alkanes. They vary in chain-length and chlorine content (30-70%) and can be divided into short-chain (SCCP, C10-13), medium chain (MCCP, C14-17) and long-chain (LCCP, C>17) CPs. These compounds are of high concern for the environment due to their persistent, toxic and bioaccumulative properties and their high production volume. Dietary intake has been shown to be a major human exposure route and more studies are now focusing on CP contamination in food (McGrath et al. 2021). However, extensive data including various food groups are still lacking and more research needs to be conducted to gain a more complete understanding of CP contamination in food. While seaweed is already widely consumed in other parts of the world, it only recently became popular as a protein alternative in Europe. Due to its high nutritional value and sustainable production, seaweed can now be found in an increasing number of products on the European market (Ferdouse et al. 2018). In its fried form, seaweed is gaining popularity as a healthy alternative to conventional potato chips, making it crucial to investigate the chemical food safety of this alternative protein source. Seaweed chips might have a higher risk of CP exposure, since both lipid content (McGrath et al. 2021) and level of processing (Yuan et al. 2017b) have been shown to be of relevance for CP contamination.

Materials and Methods: In this study, we investigated the occurrence of CPs in seaweed chips (Porphyra Yezoensis, n=4) purchased in Belgium using liquid chromatography coupled to high-resolution mass spectrometry. CP congener groups contained carbon-chains ranging from C6 to C36 and chlorine numbers ranging from Cl3 to Cl30. Homologue identification and integration were performed with the R-based open-source software "CP-Seeker" v1.1. The quantification of each CP class was conducted by chlorine-content calibration using mixtures of SCCPs, MCCPs and LCCPs (C18-20).

Results: While ∑SCCP levels were below the limit of quantification (LOQ) and ∑LCCP concentrations were low in all 4 samples (range 17-54 ng/g dry weight (dw)), ∑MCCP concentrations ranged from 34 to 799 ng/g dw and were detected in all samples. ∑MCCP was the most abundant homologue group, making up for about 80% in all samples. Sample 1 and sample 3 had the highest ∑MCCP concentrations with 419 ng/g dw and 799 ng/g dw, respectively. Due to a lack of appropriate standards, quantification of individual homologues was not possible, however, relative contributions gave information about their distribution. Among SCCPs, C13 was the most abundant homologue. The main contributor in MCCPs was C14, while for LCCPs it was C18. In the chlorine distribution, the most abundant homologues were Cl6-9.

Discussion and Conclusion: The overall CP pattern and the dominant abundance of MCCPs in the samples were consistent with previous studies in food (McGrath et al. 2021), but also with the estimated global accumulation and use of CPs. A correlation between lipid content and ∑MCCP concentrations has previously been suggested, due to the potential of lipids to accumulate CPs (McGrath et al. 2021). In addition to the lipid content, the degree of processing has been shown to be of importance for CP contamination (Yuan et al. 2017b). This compares well with our findings since seaweed chips are highly processed products with high lipid content. The detected ∑MCCP levels in two samples were higher compared with conventional chips (McGrath et al. 2021) and suggest an increased accumulation of CPs. While more research is needed to fully assess CP contamination in seaweed chips, this study offers valuable first insights and can be a starting point for future work.

References
Fate, Detection and Analysis of Chlorinated Paraffins

P-029  Interlaboratory Comparison of Medium Chain Chlorinated Paraffins Aimed for Future Development of a Reference Material

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1. Introduction:
Chlorinated paraffins (CPs), with varied chain lengths and degrees of chlorination are familiar as complex technical preparations1-3. Commercially CPs are used as metalworking fluids, plasticizers, flame retardants etc. CPs are typically divided into three groups: short-chain CPs (SCCPs) that comprise 10 to 13 carbon atoms, medium-chain CPs (MCCPs) that comprise 14 to 17 carbon atoms, and long-chain CPs (LCCPs) that comprise ≥ 18 carbon atoms1-3. In recent times, SCCPs have received considerable research attention because of their persistence and long-range transportation ability in the environment. Thus, SCCPs are registered as listed chemicals on the Stockholm Convention on Persistent Organic Pollutants (POPs). Moreover, MCCPs are discussed as restricted chemicals in regulations drafted by various countries/organizations; therefore, extensive environmental monitoring and risk assessment have been conducted for both SCCPs and MCCPs.

Because CPs have innumerable isomers1-3, accurate and precise analysis of SCCPs and MCCPs has not yet been possible3,4. Similar to SCCPs, MCCPs have been analyzed via chromatography, including gas chromatography-mass spectrometry (GC/MS) and liquid chromatography-mass spectrometry (LC/MS)4. However, comparing the analysis results with respect to SCCPs and MCCPs obtained using the existing methods is extremely difficult owing to the lack of reliable methods using reference materials5. Then, we recently performed an interlaboratory comparison of SCCPs for developing a reference material (RM). Based on these interlaboratory comparisons, NMIJ RM 4076-a, a RM with respect to SCCPs has been issued by the National Metrology Institute of Japan6.

Next, we planned an interlaboratory comparison to develop a new RM with respect to MCCPs. The first goal of these comparisons was to prepare a candidate RM with respect to MCCPs. And then, this MCCP prepared was distributed as an analytical sample for each laboratory participating in this interlaboratory comparison. This interlaboratory comparison focused on preliminary evaluations of a candidate RM with respect to MCCPs. Herein, the preliminary results obtained from this interlaboratory comparison with respect to MCCPs have been described.

2. Materials and Methods:

Interlaboratory comparison samples

The analytical sample, which can potentially be used as a candidate RM with respect to MCCPs, was employed for our interlaboratory comparison. This analytical sample was prepared by reacting pure gaseous chlorine with mixed paraffins (from C14 to C17) at ~80°C7. A mass of each paraffin was weighed by balance, and then, paraffins were mixed well each other. The reaction was terminated by stopping the chlorine flow when the desired degree of chlorination (~55 %) was attained. Then, nitrogen gas was passed over the product to remove any unreacted chlorine and residual hydrogen chloride. The final product that was obtained via pressure filtration was a candidate RM. The degree of chlorination in the final product was evaluated in terms of the mass fraction of chlorine using combustion-ion chromatography (CIC). Furthermore, impurities in the final product, such as organic matter, polymeric molecules, and water were examined using GC-FID (flame ionization detection), SEC (size exclusion chromatography), and Karl Fischer titration, respectively.

Separately, a commercially available reagent was used to prepare a quantitative standard solution decided homolog compositional ratios in advance.

Protocol of interlaboratory comparison

In this study, the abovementioned analytical sample and quantitative standard solution were distributed among the participants. All the participants reported the quantification results obtained using concentrations and homolog compositional ratios based on the quantitative standard solution.

Analytical conditions reported by participants

Results obtained from MCCP analysis were reported by 19 laboratories. Analytical equipment used for this analysis was as follows: Orbitrap MS, time-of-flight MS (TOFMS), and quadrupole MS (QMS) in GC-based instruments and TOFMS and tandem mass spectrometry (MS/MS) in the LC-based instruments. Some laboratories used GC-QMS coupled with a pyrolyzer. The different types of ionization methods used during MCCP analysis were as follows: electron ionization (EI), negative chemical ionization (NCI), and appropriate combinations of EI and NCI based on the number of chlorine atoms in GC analysis and electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) in LC analysis. Three laboratories used...
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thermal-desorption MS (TD-MS) combined with APCI. In this study, the results from these three laboratories were excluded due to the need for reanalysis. Table 1 summarizes the analytical conditions used by various laboratories. Therefore, the results related to total concentration and homolog profiles reported by 16 remaining laboratories were used in this comparison, without unifying the homologs among the participants that had reported the results related to various homolog profiles such as chlorine homologs 4–10, or 5–9. Additionally, results received from some laboratories were volume-based; were converted these results to mass-based results using density of the solvent used in the final solution. In this study, some participants reported multiple results using different analytical conditions.

Table 1: Summary of analytical conditions used by laboratories.

<table>
<thead>
<tr>
<th>Lab No.</th>
<th>Instrument</th>
<th>Ionization method</th>
<th>Target chlorination product</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>LC-MS/MS</td>
<td>ESI</td>
<td>Tetra to deca</td>
</tr>
<tr>
<td>2</td>
<td>LC-MS/MS</td>
<td>ESI</td>
<td>Tetra to deca</td>
</tr>
<tr>
<td>3</td>
<td>LC-MS/MS</td>
<td>APCI</td>
<td>Tetra to nona</td>
</tr>
<tr>
<td>4</td>
<td>LC-MS/MS</td>
<td>ESI</td>
<td>Tetra to deca</td>
</tr>
<tr>
<td>5</td>
<td>LC-MS/MS</td>
<td>ESI</td>
<td>Tetra to deca</td>
</tr>
<tr>
<td>6</td>
<td>LC-TOFMS</td>
<td>APCI</td>
<td>Tetra to nona</td>
</tr>
<tr>
<td>7</td>
<td>LC-TOFMS</td>
<td>ESI</td>
<td>Tetra to deca</td>
</tr>
<tr>
<td>8</td>
<td>LC-TOFMS</td>
<td>ESI</td>
<td>Tetra to</td>
</tr>
<tr>
<td>9</td>
<td>GC-Orbitrap MS</td>
<td>EI &amp; NCI</td>
<td>Tetra to deca</td>
</tr>
<tr>
<td>10</td>
<td>GC-TOFMS</td>
<td>EI &amp; NCI</td>
<td>Tetra to deca</td>
</tr>
<tr>
<td>11</td>
<td>GC-TOFMS</td>
<td>EI</td>
<td>Tetra to</td>
</tr>
<tr>
<td>12</td>
<td>GC-TOFMS</td>
<td>NCI</td>
<td>Penta to</td>
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<td>13</td>
<td>GC-QMS</td>
<td>NCI</td>
<td>Tetra to nona</td>
</tr>
<tr>
<td>14</td>
<td>GC-QMS</td>
<td>EI</td>
<td>Tetra to deca</td>
</tr>
<tr>
<td>15</td>
<td>Pyrolyzer-GC-QMS</td>
<td>NCI</td>
<td>Penta to deca</td>
</tr>
<tr>
<td>16</td>
<td>Pyrolyzer-GC-QMS</td>
<td>NCI</td>
<td>Penta to deca</td>
</tr>
</tbody>
</table>

3. Results: Candidate reference materials distributed as the analytical sample to participants

In this study, a candidate RM used was synthesized using gaseous chlorine and a mixture of alkanes (tetradecane, pentadecane, hexadecane, heptadecane = 1:1:1:1 mass ratio) as starting materials. This mass ratio did not change after the synthesis of SCCPs; hence, probably, the ratio could remain unchanged after the synthesis of MCCPs under nearly identical conditions. Therefore, the average number of carbon atoms was estimated to be 15.5 (C15–C16). The impurity examinations conducted in this study could not detect other organic compounds such as starting alkanes and SCCPs. Polymeric molecules such as oligomers were nearly nonexistent. The minute amount of water content (200–300 mg/kg) detected did not exhibit any influence on the candidate RM. Additionally, the mass fraction of chlorine evaluated using CIC was estimated to be 460 g/kg (46%). Hence, the average molar mass of the candidate RM was assumed to be C15H27Cl5 (46.2%) or C16H29Cl5 (45.5%).

Furthermore, the mass ratio (related to paraffins) of the quantitative standard solution was estimated to be 70:25:5:1. Therefore, the mass ratios of the analytical sample and quantitative standard solution were obviously different. Because it was difficult to purchase suitable commercially available reagents with clearly stated homolog compositional ratios. Therefore, a consideration of this concern when analysis of data was essential in our interlaboratory comparison.

Total MCCP concentration

First, values of total MCCP concentration were compared (tentative values were shown due to some laboratories reanalysing). Herein, the difference between the highest and lowest values of MCCP concentration was approximately four times the lowest value because of the large difference between the homolog compositional ratios related to the analytical sample and quantitative standard solution. A coefficient of variation (CV) with respect to the data obtained from 16 laboratories was ~45%. Based on the equipment used for analysis, the results obtained from high-resolution MS such as Orbitrap MS and TOFMS showed a difference of about three times (CV 34%) among 7 laboratories. Herein, the results obtained using GC-QMS (including
GC-QMS coupled with a pyrolyzer) were nearly comparable to those obtained using the high-resolution MS. This was because of the difference between the impact of GC-based and LC-based instruments on interferences to MCCP analysis. Because MCCPs interfered with each other when analyzed using LC-based instruments, the effect of these interferences was not negligible; thus, a large difference was observed.

**MCCP carbon chain length profiles**
Figure 1 shows the carbon chain length profiles. Some laboratories reported highly varied results; however, results from most laboratories were consistent and resembled the mass ratio of starting paraffins (1:1:1:1). Results from most laboratories showed that C14 was present in large proportions in MCCPs and C17 was present in small proportions (especially when analysis was conducted using GC-based instruments). This trend was attributed to the mass ratio of paraffins (70:25:5:1) in the quantitative standard solution. Although this trend should be observed in results obtained using all the instruments because all the laboratories used the same quantitative standard, the trend was particularly evident in the results obtained using GC-QMS coupled with a pyrolyzer. In most results obtained using high-resolution MS and GC-based instruments, the proportion of C14 in MCCPs might be high. Certain trends may appear based on different methods used for analysis; thus, further survey is needed.

**Figure 1:** Relative carbon chain length profiles reported by laboratories in this study.

4. **Discussion:**
In this MCCP study, the CV with respect to the data obtained from 16 laboratories was equivalent to that obtained from other interlaboratory comparisons involving SCCPs8-10. Similar to the results obtained from these other interlaboratory comparisons involving SCCPs, the similarity between the homolog compositional ratios related to the analytical sample and quantitative standard was essential. Additionally, the results obtained herein using GC-based and LC-based instruments exhibited sample dependence (advantages and disadvantages) similar to that exhibited by results reported in previous studies11,12. The mutual interference among MCCPs can be an issue related to the general-purpose equipment using LC-based instruments. Moreover, similar to the results from the interlaboratory comparisons involving SCCPs13-15, the results obtained using high-resolution MS varied marginally from those obtained using general-purpose equipment for the analyses. Specifically, results obtained using high-resolution MS were more reliable than those obtained using the general-purpose equipment for analyses.

Mass fraction of chlorine (460 g/kg) in this analytical sample was verified using CIC. Moreover, we tried to verify the degree of chlorination using the concentration of each homolog, as reported in our previous report6.

5. **Conclusions:**
First, an evaluation of the candidate RM was conducted for an interlaboratory comparison of MCCPs. To improve the reliability of this candidate RM, additional impurity investigation might be required. This comparison showed substantial variations in
the results related to the concentrations and homolog profiles of MCCPs because the homolog compositional ratios related to the analytical sample and quantitative standard did not resemble in case of MCCPs. By contrast, the results obtained using different types of high-resolution MS, including GC-Orbitrap MS, GC-TOFMS, and LC-TOFMS showed small variations with respect to MCCP analysis. Despite the lack of interferences spiked in this analytical samples, the results obtained using the general-purpose equipment (GC and LC) exhibited some advantages and some disadvantages.

To develop an RM with respect to MCCPs, another interlaboratory comparison will be planned in near future. Simultaneously, we intend to conduct a highly detailed survey to analyze the obtained results with respect to chlorine homologs (data not shown) in this MCCP analysis.

6. Acknowledgments:
This work was partially supported by JSPS KAKENHI Grant-in-Aid for Scientific Research(C) (Grant Number JP21K12285). We appreciate Dr. Keisuke Nakamura from NMIJ/AIST for supporting in performing SEC of the candidate RM. The CIC for the evaluation of the mass fraction of chlorine on the candidate reference material was performed at the Tokyo Metropolitan Industrial Technology Research Institute (Japan). We would like to thank the 16 participants that joined in this study: Tohoku Ryokka Kankyohozan; Shimadzu Techno-Research; National Institute for Environmental Studies; IDEA Consultants; Agilent Technologies; Chiba University; MIURA; Hyogo Prefectural Institute of Environmental Sciences; NIPPON STEEL TECHNOLOGY; TOSHIBA; Environmental Control Center; Environmental Research & Solutions; FUJI FILM; Brother Industries; Hitachi High-Tech Corporation; Eurofins Genomics.

7. References:
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**Fate, Detection and Analysis of Chlorinated Paraffins**

**P-031  Estimating SCCPs Emissions in Japan: Role of Imported Products and MCCPs Impurities**

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**Introduction:** While previous studies (Tsunemi, 2010; Gluge, et al., 2016; Koshiba, et al., 2023) have estimated emission inventories of short-chain chlorinated paraffins (SCCPs), they overlooked the impact of imported products containing SCCPs and SCCPs present as impurities in medium-chain chlorinated paraffins (MCCPs). This study aims to quantify SCCPs emissions in Japan, including these overlooked sources.

**Materials and Methods:** We calculated the SCCPs material flow and emission at each lifecycle stage of the products from 1990 to 2050. Our estimates encompass both locally produced SCCPs and those present as impurities in MCCPs. We presumed a 1% impurity rate of SCCPs in MCCPs by weight, and also factored in SCCPs present in imported polyvinyl chloride products. To validate our results, we predicted environmental SCCPs concentrations using G-CIEMS, which is the environmental fate model having high spatial resolution (developed by National Institute of Environmental Studies, Japan). Spatial distribution of SCCPs emission was assumed to be in proportion to population distribution and industrial production value.

**Results:** Peak atmospheric total-SCCPs emissions were observed in 1992 and 1993 at approximately 20 tonnes/y (Fig.). Subsequently a declining trend in SCCPs emissions was observed. Initially, the largest contribution came from the long-term use of domestically produced products, but recently, the emissions from long-term use of imported products have been rising. The emissions from SCCPs as MCCP impurities were consistently less than one tonne/y. The predicted atmospheric total-SCCPs concentration in 2019 was approximately 70 pg/m³ on average and 3,000 pg/m³ in some populated areas. The predictions and observations agreed well in eastern Japan, but the predictions tended to be lower than the observations in western Japan. The prediction of atmospheric total-SCCPs concentration was improved compared to that predicted by Koshiba, et al., 2023.

**Discussion and Conclusion:** Our total-SCCPs emission estimates are 6.7 tonnes/y in 2020, which was higher than those estimated by Koshiba, et al., 2023 (approx. 4 tonnes/y) due to our inclusion of imported goods and MCCP impurities. In conclusion, it was suggested that SCCPs as impurities in MCCPs would not be a significant emission source, on the other hand, SCCPs contained in imported products could be an important emission source in the future.

**Fig.** Estimation results of atmospheric total-SCCPs emission

**References:**
Fate, Detection and Analysis of Chlorinated Paraffins

P-032 Occurrence of Polychlorinated Alkanes in Animal Feed from the German Market

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Introduction: Short chain chlorinated paraffins (SCCPs) are currently listed in Annex A of the Stockholm convention, while the corresponding medium chain CPs (MCCPs) are listed in the Candidate List of Substances of Very High Concern under Reach Regulation. Amongst all potential exposure routes for humans, dietary intake of chlorinated paraffins (CPs) is considered to be the main contribution for adults and thus well studied, but little is known about CP levels in animal feed. Likewise, intake of CPs from feed, especially fatty additives, may be a major exposure route for CPs in animals. Therefore, more information on CP levels in feed is needed to assess the probability of carry-over effects from contaminated feed to food. Furthermore, comparison of homologue patterns from feed and the corresponding animal products may provide insight into animal metabolism. Until now, we are not aware of any other studies regarding this topic, except for one[1] that covers samples from China which might differ significantly compared to findings for Europe due to restrictions for use in production. We thus present first results of an ongoing effort to assess CP levels in feed.

Materials and Methods: Samples were acquired though the official sampling plan of Baden-Württemberg for the analysis of PCDDs/Fs and PCBs in feed. Since CPs are presumed to accumulate in fatty matrices, only samples with a fat content of more than 5% were considered, including e.g. various fatty feed additives on the basis of rapeseed, soybeans or linseed, milk replacements and fish feed. Sample preparation followed a well-established procedure including cold solvent extraction for the isolation of CPs from the sample with n-hexane/DCM (1/1, v/v) followed by an acid silica column purification step for matrix separation. Further separation of other chlorinated POPs was done by a magnesium silicate column. Analysis of the extracts was carried out with a Thermo Fisher Q Exactive Orbitrap in NCI mode (mass resolution 120000), equipped with a DB5 column for GC separation.[2] Within all CPs, polychlorinated alkanes (PCAs) are the most abundant and best studied class, due to the availability of suitable standards for quantitative analysis, and their levels are often taken as indicative representatives for total CP content of a sample. The high mass resolution of the Orbitrap allows to unambiguously identify and quantify short- and medium-PCAs. Longer species are not accessible due to high vaporization energies. Chlorinated olefins were not quantified due to a lack of suitable standards.

Results: The study is planned as a continuous effort, therefore only preliminary results will be presented. First findings indicate presence of PCAs in all fatty materials with levels between 5 and 500 ng/g fat. Both, S- and MCCPs were present in all samples, with SCCP levels ranging from <1 to 140 ng/g fat and MCCPs from 6 to 500 ng/g fat. A linseed based additive for horses showed a very high contamination of >9000 ng/g fat, which included S- and MCCPs in a ratio of 1:2. Most congener patterns showed a typical unimodal Gaussian distribution of chlorine atoms for one chain length but some exhibited broader, also bimodal distributions, potentially indicating multiple sources of contamination or metabolic effects. C11 was found to be dominating for SCCPs and C14 for MCCPs.

Discussion and Conclusion: Findings of CPs in all analyzed samples, especially at higher levels in oils, indicate that further studies are needed to generate substantially more data and assess the overall contamination situation of feed materials with CPs. The overall strong dominance of MCCPs over SCCPs, detrimentally opposed to findings from China,[1] might result from the current EU legislation effectively banning SCCPs from use and production.

Acknowledgements
We would like to thank the European Commission for the financial support of the work of the European Union Reference Laboratory for Halogenated POPs in Feed and Food, Freiburg, Germany.

References:
Introduction
The environmentally damaging and socially detrimental organics, polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs), are always unintentionally emitted from municipal solid waste incinerators (MSWIs)\(^1,2\). The chemical inhibitor addition (including nitrogen-, sulfur-, phosphorus- and calcium-based compounds), one of the source inhibition technology, is widely employed to control the PCDD/F emission and shows significant inhibition efficiencies\(^3-7\).

Compared to N-, S-based inhibitors, CaO or Ca(OH)\(_2\) is more economic and eco-friendly with the low toxicity and strong adsorption of acidic gases such as HCl, Cl\(_2\), and SOx (decrease the risk of equipment corrosion)\(^8\). However, previous studies of CaO or Ca(OH)\(_2\) inhibition on PCDD/F formation concentrated on the high-temperature zone and precursor formation. For example, Ca(OH)\(_2\) was used as an upstream sorbent for HCl and Cl\(_2\) reduction in waste combustors (>800\(^\circ\)C) and effectively decreased the PCDD/F emission (over 90%)\(^9\). CaO employed on the PCDD/F formation from the pentachlorophenol, and recud over 90% PCDD/F yields 10. Limited studies paid attention on CaO-induced inhibition of PCDD/Fs by \textit{de novo} synthesis. Also, previous studies manifested that CaO inhibited PCDD/F formation mainly by adsorbing the HCl and Cl\(_2\) (Cl source) 8,9. Whereas, the explicit insight into the evolution of calcium and the detailed effect of CaO on copper compounds for \textit{de novo} synthesis are scarce.

To fill this gap, we conducted a laboratory study in the temperature of 250-450\(^\circ\)C focusing on the inhibition effect of CaO on PCDD/Fs formation and the PCDD/F homologues by \textit{de novo} synthesis. The morphology-dependent analysis concerning the chemical speciation variation of major elements (C, Cl, Cu) in model fly ash and the crystal structure conversion of CaO and CuCl\(_2\) was conducted to probe into the inhibition mechanisms of CaO. Regarding the low price and low toxicity of CaO, it is expected to apply in real MSWI and the results of this study also are aimed to provide theoretical support for the practical application.

2. Materials and method
2.1 Sample preparation
The model fly ash (MFA) consisted of SiO\(_2\) (91.6\%), activated carbon (AC, 3\%), NaCl (5\%) and CuCl\(_2\).H\(_2\)O (0.4\%)\(^5\), stored in a sealed bag under the dry and dark conditions. Two kinds of CaO (AR, purity>98\%) were used as PCDD/F inhibitors, noted as CaO\(_{\text{a}}\) (\(\approx 4.8\) \(\mu\)m) and CaO\(_{\text{b}}\) (\(\approx 8.4\) \(\mu\)m), respectively. 1~5\% CaO was added in MFA samples. In addition, all the chemical reagents used in the collection, extraction, purification and concentration of PCDD/F, including toluene, hexane, acetone, dichloromethane, and XAD-2 are of chromatographic grade (Fisher Scientific, USA).

2.2 Pre-treatment procedures and PCDD/F analysis
The formation and inhibition experiments of PCDD/Fs are conducted in a vertical tubular furnace. The reaction chamber was preheated to a specific temperature (250~450\(^\circ\)C), and then, the samples was placed in the chamber for 30 min, accompanied by the model flue gas (88 vol.\% N\(_2\)+12 vol.\% O\(_2\), 100 mL/min\(^11\)). In off-gases, the gas-phase PCDD/Fs were absorbed by 7 g XAD-II resin and 200 mL toluene. After each experiment, the solid residual, XAD-II resin and toluene, and the toluene eluent of the reaction chamber were collected. All the collected samples were pretreated (consisted of extraction, purification, and concentration procedures) based on the US EPA 1613 standard method\(^12\), and then analysed by a high-resolution gas chromatography-mass spectrometry (HRGC/MS, JMS-800D, JEOL Co., Japan). And the recoveries of PCDD/F standards ranged from 32 to 133\%, achieving the requirements of the US EPA 1613 method.

2.3 Morphological characterization analysis
The valence state of the surface elements (chlorine, carbon and copper) of solid samples was determined by X-ray Photoelectron Spectroscopy (XPS, Thermo Scientific K-Alpha, USA). The binding energy of 284.8 eV (contaminated carbon) was applied to calibrate the binding energies of other elements. The XPS spectra of Cl\(_2\)p, Cl\(_{1}\)s and Cu\(_{2}\)p were then deconvoluted using the software of Avantage based on the Shirley-type baseline and an iterative least-squared optimization algorithm. In situ variable temperature XRD (Rigaku Smartlab, Japan) was employed to provide the conditions close to the real conditions (200~450\(^\circ\)C), investigating the phase evolution of samples (CuCl\(_2\).H\(_2\)O+CaO+AC) during the reaction process.

3. Results and discussion
3.1 The inhibition effect of CaO on PCDD/F concentrations and homologous
Figure 1 displays the overall PCDD/F concentrations and the inhibition efficiencies of CaO within the temperature range of 250~450\(^\circ\)C. The addition of CaO showed considerable inhibition effects on PCDD/F formation. The PCDD/F concentrations reduced from 2272.01 to 55.17~746.26 ng/g, together with the I-TEQ concentrations decreased from 12.01 to 0.48~8.71 ng I-TEQ/g at 350\(^\circ\)C. During the process of PCDD/F generated from fly ash, CaO performed a notable inhibition effect (especially at 250 and 350\(^\circ\)C, the inhibition efficiencies over 90\% with 5\%~10\% CaO). The addition of CaO (1~10\%) during 250~450\(^\circ\)C significantly
inhibited the generation of HCDD/Fs (51.5~99.8%) and OCDD/Fs (85.6~99.8%), while the inhibition efficiencies were -106.8, -99.3 and -13.11% for TCDD of 5Ca-MFA(350°C), TCDF of 1Ca-MFA(450°C) and TCDF of 10Ca*-MFA(450°C), respectively. Thus, it was found that CaO was more prone to inhibit the formation of hepta- and octa-CDD/Fs than tetra- to hexa-CDD/Fs, which perhaps be attributed to the dechlorination effect of CaO on PCDD/F-congeners.

Figure 1. PCDD/F concentrations and the inhibition efficiencies with the addition of CaO during 250~450°C.

3.2 Inhibition mechanisms
The XPS analysis (Figure 2a-c) showed that CaO promoted the formation of inorganic chloride (from 83.5% to 88.7~93.5%) and inhibited the formation of organic chloride (from 16.5% to 6.5~11.3%), which suggested that the organic Cl was converted to inorganic Cl. CaO caused the growth of Cu2O and CuO (from 26.3% to 32.9~33.3%) but the reduction of CuCl2 (from 11.8% to 3.2~4.9%), manifesting that CaO promoted the conversion of CuCl2 to CuO/Cu2O (the deactivation of CuCl2) corresponding to the previous study 8. Moreover, CaO prominently decreased the proportion of C=C (from 6.7% to 1.3~2.1%), probably indicating the inhibition effect on the formation of unsaturated hydrocarbons or aromatic carbon. Additionally, the proportion of C-O/C-N decreased from 10.1% to 6.9~8.1% with the addition of CaO, perhaps suggesting the inhibition of the formation of CO or the hydrocarbon that contains the C-O group. The CaCO3 generation was reflected by the increase of -COOR/CO32- (from 6.4% to 11.8~12%), which could be validated by XRD analysis.

The conversion of CaO and CuCl2 was performed by the in situ variable temperature XRD (Figure 2d). From 200 to 250°C, the signal intensity of CuCl2, Cu2Cl(OH)3, CuCl and CaO weakened, and a small amount of CaCl2, CaCO3 and CuO was produced. From 250 to 350°C, copper chlorides (CuCl2, Cu2Cl(OH)3, and CuCl) gradually disappeared, and intensive signals of CaCl2, CaCO3 and CuO appeared. The reaction processes and the species evolution could be summarized as follow: CuCl2.2H2O was primarily dehydrated to CuCl2 and part of was transferred to Cu2Cl(OH)3 at 200°C, while CaO combined with the lost crystal water to form Ca(OH)2; Meanwhile, Cu(II)Cl2 was partly reduced to Cu(I)Cl by the reductant C or CO 13, and CuCl was subsequently oxidized and dechlorinated to CuO with the participation of CaO/Ca(OH)2 (350~450°C), simultaneously accompanied by the production of CaCl2; Also, Ca(OH)2 adsorbed CO2 to form CaCO3. Also, our previous study investigated the TG-FTIR of MFA and MFA+CaO shown in Figure 2 e-f 14. The weight loss rate remarkably decreased after MFA adding with CaO starting from about 340°C (Figure 2e), and it could be attributed to the formation of CaCO3 in terms of both the DTG and XRD analysis. According to the FTIR spectra (Figure 2f), CaO considerably reduced the generation of the PCDD/F-related organic compounds consisting of -C=H on the benzene ring and C=O (at 350°C), indicating the inhibition effect on de novo synthesis.
4. Conclusions

In this study, laboratory experiments during 250~450°C were carried out to investigate the inhibition effect of CaO on PCDD/F formation for the practical employed in real MSWIs, and morphological analysis (XPS and XRD) were conducted to further excavate the CaO-induced inhibition mechanisms on de novo synthesis. Main conclusions were as follows:

(1) CaO significantly inhibited the PCDD/F formation during 250~450°C and inhibition efficiencies reached up to over 95% with the addition of 10% CaO. Results showed that the addition of 5~10% CaO and the finer particle (~4.8 μm) inhibited over 90% PCDD/F concentrations and I-TEQ concentrations (250 and 350°C), which provides the proper employment conditions in real MSWIs.

(2) CaO promoted the dechlorination effect by: i) the high chlorinated PCDD/F-congeners converted to low chlorinated; ii) the superficial organic Cl reduced from 16.5% to 6.5~11.3%.

(3) CaO facilitated the passivation of CuCl2 (CuCl2 converted to CuO), and inhibited the chlorination of the carbon matrix and solidified the Cl source. Also, the formation of unsaturated hydrocarbons or aromatic carbon (superficial C=C reduced from 6.7% to 1.3~2.1%) during de novo synthesis was suppressed.
5. Acknowledgment
This study was supported by the Innovative Research Groups of the National Natural Science Foundation of China (51621005).

References
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1. Introduction
The Japan Environmental Safety Corporation (JESCO) has adopted a chemical-based treatment of polychlorinated biphenyl (PCB) waste¹ and has been operating five PCB waste treatment facilities across Japan since 2004. As the only operator of a high-concentration PCB waste treatment facility in Japan, the corporation has been promoting the project to realise the completion of treatment, emphasising the safe and reliable implementation of the project and information sharing and disclosure. In the near future, dismantling and removal will be conducted at each facility at an appropriate time after the treatment of PCB waste is completed and operations will be terminated. The Phase I facility at Kitakyushu Works ceased operations at the end of March 2019 and has already entered the dismantling and removal phase. It is assumed that the equipment and piping may need to be cut using plasma cutting technology during demolition and removal. Therefore, the effects of plasma cutting of PCB-contaminated materials on the working environment concentrations of PCBs and PCDDs/PCDFs should be investigated. In addition, the thermal transformation of PCBs adhered to dioxins under high-temperature plasma cutting is also examined.

2. Materials and Methods
2.1 Simulants preparation
The surfaces of SUS316 (stainless steel) of H100 cm x W200 cm x T9 mm and CS (carbon steel) of H90 cm x W180 cm x t9 mm were coated with a PCB adjustment liquid of seven levels at PCB concentrations of 0~9,720 ppm to simulate equipment and piping contaminated with PCBs. The PCB adjustment liquid was prepared by diluting PCB oil (the original solution before adjustment), from which PCB oil extracted from the transformers was distilled to remove trichlorinated benzene with a paraffin solvent (trade name: NS Clean, n-C12H26). The concentration of the PCB on the simulant surface was measured using a wiping test.

2.2 Plasma cutting operations.
An M-5500CII (inverter air plasma) manufactured by DAIHEN Co., Ltd., was used as the plasma cutting machine; its cutting speed was approximately 60 cm/min, and the simulated objects were cut at 5 cm intervals in the short-axis direction for 5 min, followed by a 5-min break. During the cutting operation, the suction port of the local exhaust system was placed 10–20 cm from the cutting area (trapping distance), and the surrounding gases, including fumes generated by cutting, were constantly sucked at a suction rate of 5 m³/min. Gases in the local exhaust system were collected and analysed for PCBs and DIOXINs (polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and dioxin-like PCBs (dl-PCB)).

2.3 Measurement of working environment concentrations (i.e., PCBs and DIOXINS)
During plasma cutting operations, gas sampling with a high-volume sampler was conducted in the vicinity of the cutting operator to determine the working environment concentrations of the PCBs and DIOXINS.

2.4 Location and duration of implementation
The project site was the Kitakyushu PCB treatment works, phase 1 facility, with a period of 11-22 May 2021.

3. Results and discussion
3.1 Surface PCB concentrations in simulated materials and working environment
Table 1 lists the working environment concentrations (PCBs and DIOXINS) with different surface PCB concentrations when plasma cutting was performed with local exhaust ventilation installed.
Formation, Sources and Control

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<table>
<thead>
<tr>
<th>Simulated Object No.</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steel plate material</td>
<td>SUS</td>
<td>SUS</td>
<td>CS</td>
<td>SUS</td>
<td>SUS</td>
<td>SUS</td>
<td>SUS</td>
<td>CS</td>
</tr>
<tr>
<td>Conditioned solution PCBs concentration (mg/kg)</td>
<td>0</td>
<td>183</td>
<td>243</td>
<td>612</td>
<td>1,223</td>
<td>3,670</td>
<td>7,339</td>
<td>9,720</td>
</tr>
<tr>
<td>Wiping test (µg/100cm²)</td>
<td>0</td>
<td>31</td>
<td>24</td>
<td>65</td>
<td>140</td>
<td>570</td>
<td>1,100</td>
<td>950</td>
</tr>
<tr>
<td>PCBs in the working environment (µg/m³)</td>
<td>0.18</td>
<td>0.23</td>
<td>0.16</td>
<td>0.35</td>
<td>0.65</td>
<td>0.97</td>
<td>1.9</td>
<td>1.1</td>
</tr>
<tr>
<td>Working environment DIOXINs (pg-TEQ/m³)</td>
<td>0.50</td>
<td>3.1</td>
<td>1.5</td>
<td>4.6</td>
<td>9.8</td>
<td>11</td>
<td>46</td>
<td>6.5</td>
</tr>
</tbody>
</table>

Table 1 Surface PCB concentrations in simulated materials and working environment concentrations

The PCBs and DIOXINs present as gaseous and particle phases in the working environment and the local exhaust system at the time of cutting simulant No. 6 are listed in Table 2. The working environment control concentration of the PCBs was 0.01 mg/m³ (10 µg/m³), which was below the working environment control concentration of the PCBs in all cases when the simulants were reduced (Table 1). On the other hand, the working environment control concentration of DIOXINs was 2.5 pg-TEQ/m³, which was satisfied for simulants No. 1 and No. 2 but exceeded in all other cases. The relationship between the PCB concentrations in the wipe test and DIOXINs concentrations in the working environment is represented as follows: y = 0.037x + 0.4652 (R² = 0.8938). Using this formula, the PCB concentration in the wiping test corresponding to a working environment control concentration of 2.5 pg-TEQ/m³ was determined to be 55 µg/100 cm², but we considered that plasma cutting could be applied when the concentration was less than 30 µg/100 cm², to further ensure safety.

Table 2 Fraction of gaseous and particulate PCBs and DIOXINs

As shown in Table 2, the dust content in the local exhaust system was twenty times higher than that in the working environment, and the particle phase (fumes) generated during the plasma cutting of the simulants was effectively removed by the local exhaust system. DIOXINs are also present in these particle phases, and their release into the working environment during plasma cutting can be reduced by the appropriate use of local exhaust ventilation systems. Based on the above, plasma cutting is applicable when the surface PCB concentration of the cutting object is less than 30 µg/100 cm² (wipe test) and under proper installation of a local exhaust ventilation system.

3.2 Thermal transformation of PCBs

The results of the study on the fractions of PCDDs, PCDFs, and dl-PCB formation in the total TEQ (T-dioxins) under the conditions of Simulant No. 6 are presented in Table 3. Compared to the high fraction of PCDFs (77%) in Kanemi rice oil (2-5), which was produced by the exposure of PCBs to high temperatures, the fraction of PCDFs in the working environment at the time of cutting simulant No. 6 was low, specifically at 7.8%. This value is comparable to the proportion of PCDFs (3.2%) in the pre-conditioned raw materials used to create the simulant, suggesting that the DIOXINs identified in the working environment during plasma cutting were not produced by the thermal transformation of PCBs but by evaporation of the applied PCBs. Furthermore, the proportions of 2,3,4,7,8-PeCDF, 1,2,3,4,7,8-HxCDF and 3,3',4,4',5-PeCB in the total TEQ (T-dioxins) are shown in Table 4. Compared to the high fraction of 2,3,4,7,8-PeCDF in Kanemi rice oil (56%), its low fraction (2.7%) in the working environment during the plasma cutting of simulant No. 6 is potentially indicative of there having been no thermal transformation of PCBs.
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The proportions of the three isomers, namely, 3,3',4,4'-TeCB (#77), 3,3',4,4',5-PeCB (#126) and 3,3',4,4',5,5'-HxCB (#169) have been studied in municipal solid waste incineration fly ash; they were found to be 50%, 40%, and 10% respectively. Furthermore, #169 is rarely present in PCB formulations and is produced through combustion. In the working environment, during the plasma cutting of simulant No. 6, #77, #126, and #169 were 88.4%, 11.3%, and 0.3%, respectively, with a low proportion of #169, which is produced through combustion. Considering this perspective, there seems to be no thermal transformation of the PCBs. The proportions of the three isomers in the incinerated fly ash are listed in Table 5.

Table 5 Proportion of three isomers present in incinerated fly ash

<table>
<thead>
<tr>
<th>#77 : #126 : #169</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCBs preparation</td>
</tr>
<tr>
<td>99 : 1 : 0.006</td>
</tr>
<tr>
<td>Pre-conditioning</td>
</tr>
<tr>
<td>solution</td>
</tr>
<tr>
<td>93.2 : 6.3 : 0.4</td>
</tr>
<tr>
<td>Incinerator fly ash</td>
</tr>
<tr>
<td>50 : 40 : 10</td>
</tr>
<tr>
<td>Simulated object 6 (working environment)</td>
</tr>
<tr>
<td>88.4 : 11.3 : 0.3</td>
</tr>
</tbody>
</table>

4. Conclusions
We found that if the concentration of surface PCBs was below 30 µg/100 cm² (i.e., as determined via a wipe test) under properly installed local exhaust ventilation, plasma cutting technology can be used as a demolition and removal technology for PCB treatment facilities; additionally, this technology can be applied used without the stipulated working environment concentrations of PCBs and DIOXINs being exceeded. These results are reflected in the manual prepared by JESCO for dismantling and removing PCB waste treatment facilities.

References.
Introduction

A widely manufactured industrial chemical, polychlorinated naphthalene (PCN) formulations (also referred to as commercial or technical mixtures) were synthesized by the catalytic chlorination of molten technical naphthalene with chlorine gas. PCNs were manufactured in several countries between 1910~1980, but there is no reliable data on the volumes produced by manufacturers or national outputs for this period. Technical PCN mixtures, e.g. Halowaxes, always contain some minor by-products such as naphthalene, chlorophenols, chlorobenzenes, polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), as impurities (Noma et al., 2004a, 2004b, 2004c, 2004d, 2005a, 2005b).

These mixtures were used in a range of diverse applications worldwide. Since the 1930s, some of the PCNs were replaced with polychlorinated biphenyl (PCB) mixtures.

PCNs were used principally as dielectrics, impregnants and flame retardants in electric cables and cable sheath insulation, automotive and other capacitors. PCNs were also used for dispersive applications in adhesives, cutting, hydraulic and grinding fluids, engine oil additives, electroplating masking compounds, feedstocks for dye manufacture, filtering aid, flame (fire) retardants, fungicides, impregnant for carbon electrodes used for chlorine production, impregnating/waterproofing agent, insecticide, lubricant, mole repellent, plastic and rubber additives, proprietary uses in fabrics, refractive index testing oils, sealants, solvent, stabilizer, termicide, waxes for casting moulds, vulcanizing agent or wood preservative and some military applications. By the 1980s, the major producers (Europe excluding Russia, USA, Japan) voluntarily stopped production although the use of PCNs, particularly in closed applications continued. One known episode of a somewhat wider use of approximately 18 tons of stockpiled PCNs was an import into Japan during 1998-2000, from the DuPont Dow Elastomers company based in the UK (Yamashita et al., 2003; Falandysz et al., 2008; Yamamoto et al., 2018).

Since December 15, 2016, the amended Annexes A (elimination) and C (unintended production) to the Stockholm Convention, list most of the PCN compounds (UNEP, 2017 and 2019), with the exception of two monoCNs. MonoCN (1-chloronaphthalene; melting point -2.3°C is used as a solvent and an intermediate and is in continuous production and sale, e.g. by India (Indiamart, 2023; ILO, 2023).

The historical (1910-1970s) production and resulting emissions through diffusive/evaporative releases through usage, are still reflected in documented occurrence and patterns of PCNs in human milk in Europe and other locations worldwide (Tschiggfrei et al., 2023). More recently, PCN occurrence in human milk in some Chinese provinces has been linked to local unintentional industrial emissions (Li et al., 2020). This review updates information on the historical manufacture and unintentional production of PCNs.

Manufacture and use of PCNs in Poland

Relative to the amounts manufactured globally, a small amount of PCNs was also produced in Poland. The annual production was 15 tons of 1-Chloronaphthalene and 9 tons of the higher chlorinated (tri- to octa-CN) Woskol mixture (Biatas & Szymanowski, 2000). Thus, the total volume of PCNs manufactured during 1936-1939 in Mościce was 47 tons of 1-chloronaphthalene and 36 tons of Woskol.

In the years 1947-1968, the Mościce Chemical Plant was partly rebuilt from the damage sustained during WWII. This was accompanied by repatriation from Germany of some (6,400 tons) of the stolen machine park equipment. Thus, the manufacture of chlorine and some chlorinated chemicals was reactivated (Biatas & Szymanowski, 2002). Undoubtedly, the manufacture of 1-chloronaphthalene and Woskol was also resumed, because they were used in a product named Xylamit - a wood preservative formulation, which was produced during ~1950-1987. The book by Biatas and Szymanowski (2002) does not provide any information on the annual or total volume of PCNs manufactured in the Mościce plant when production resumed after WWII. Woskol was considered as a deficit product for the manufacture of Xylamit and it can be reasonably assumed that the annual production of 1-chloronaphthalene and Woskol was of the same size as before the war. Thus, the estimated total output in the
period of 1951–1987 was 369 tons for Woskol and 602 tons for 1-chloronaphthalene.

**Unintentional production of PCNs due to manufacture of PCBs (Chlorofen and Tarnol) in Poland**

PCBs were manufactured in Poland under the formulation product names of Chlorofen and Tarnol. Chlorofen, a highly chlorinated product used as a grease in the mining industry, was manufactured during 1966–1970 at a total volume of 1,000 tons (Falandysz & Szymczyk, 2001). 679 tons of Tarnol were manufactured from 1971 to 1976 in the Zakłady Azotowe in Mościce, Tarnów (Falandysz & Szymczyk, 2001; Sułkowski et al., 2003).

Considering that the typical PCN content of Chlorofen was 408 mg kg⁻¹ (Taniyasu et al., 2005) the total PCN content in the Chlorofen manufactured in Poland has been estimated as 0.408 tons. In 97.5% of the product, this by-production was composed of octa-CN followed by hepta-CNs with traces of hexa-CNs (Yamashita et al., 2000; Falandysz, 2007). Tarnol (ca. 40% chlorine per biphenyl molecule) had a physical appearance and properties that were similar to other well-studied PCB formulations such as Aroclor 1248, Clophen A 40, Clophen T 64, Delor 104, Phenoclor DP-4 or Kanechlor KC-400. The PCN content of Aroclor 1248 was 43.3 mg kg⁻¹, Clophen A 40 - 102.3 mg kg⁻¹, Clophen T 64 - 196 mg kg⁻¹, Delor 104 - 460.3 mg kg⁻¹ and Kanechlor KC-400 - 33.5 mg kg⁻¹ (Yamashita et al., 2000; Taniyasu et al., 2003; Ishikawa et al., 2005). Thus, the estimated content of PCNs in the total volume of manufactured Tarnol could lie between 0.023 and 0.31 ton. For purpose of comparison, estimated by-production of PCNs (Yamashita et al., 2000; Taniyasu et al., 2003) during the manufacture of other PCB formulations was: 16.969 tons (USA) for the Aroclors, 2.063 tons (Great Britain) for Seekay wax, 11.737 tons (Germany) for Clophens, 3.677 tons (Czechoslovakia) for Delors, 60.1 tons (France) for Phenoclors, 4.996 tons (Japan) for Kanechors and 73 tons (Soviet Union/Russia) for Sovol. In China, manufacture of the PCB-3 formulation resulted in the by-production of 7.3 tons of PCNs (Huang et al., 2015).

All the waste material (total volume of 90 tons) obtained after distillation of crude Tarnol was most probably used in the wood preservative product, as mentioned, using 1-chloronaphthalene, Woskol and other chemicals. These wastes consisted of a highly chlorinated, dark, PCB product (Sułkowski et al., 2003) which was not characterised. The PCB formulations with high chlorine content (probably all of distilled grade) such as Aroclor 1260, Aroclor 1262, Clophen A 60, Delor 106, Kanechlor KC-600, Sovol and Phenoclor DP6 showed PCN by-production at concentrations of 67.2 mg kg⁻¹, 65.8 mg kg⁻¹, 42 mg kg⁻¹, 147 mg kg⁻¹, 106.1 mg kg⁻¹, 730.8 mg kg⁻¹ and 227.5 mg kg⁻¹, respectively (Yamashita et al., 2000; Taniyasu et al., 2003). Thus, the estimated content of PCNs in the total volume of the waste material used to produce Tarnol could range from 0.0059 to 0.066 ton.

**Unintentional production of PCNs due to thermal sources or the use of chlorine**

In addition to the known source of unintentional formation during PCB production, PCNs are also inadvertently produced when chlorine gas is used in the manufacture of other industrial chemicals e.g., chloroparaffins, chlorinated methanes, chlorobenzene, trichloroethylene or tetrachloroethylene, and in other processes and products using chlorine gas, such as the chlorination of tap water, and in the chloro-alkali industry (Fernandes et al., 2017; Liu et al., 2021). Additionally, in much the same way that polychlorinated dibenzo-p-dioxins and furans (PCDD/Fs) are formed, PCNs are also generated during: combustion processes such as the incineration of organic waste materials (municipal, medical and hazardous wastes); fossil fuels and biomass fuel combustion; forest and other fires; ore and scrap metal smelting and refining (aluminium, copper, iron, lead, magnesium, zinc, steel production using electric arc furnaces, cement production and coke production (Liu et al., 2014).

The mechanisms that govern the formation of PCNs in these thermal sources are considered to be similar to those that are responsible for the formation of PCDD/Fs, but the compositional patterns of PCN homologues (and to a lesser extent, the congeners) that are formed, differ, depending on the type of source. The emissions of PCNs from multiple thermal sources are characterized by the co-occurrence of 1,2,3-triCN, 2,3,6-triCN, 1,2,3,6-tetraCN, 1,3,6,7-tetraCN and 1,2,3,6,7-pentaCN which are absent or very minor constituents in technical PCN and PCB formulations, but occur in the global environment (Orlikowska et al., 2009).

Among waste incineration sources, PCNs found in flue gas and on fly-ash were found to be higher in medical and industrial waste incinerators than municipal incinerators (MSWIs) (Li et al., 2015). In most of the thermal sources, the PCN toxic equivalents (TEQs) were lower than those of PCDD/Fs (Li et al., 2017; Liu et al., 2013), but for cement kilns co-processing solid wastes, the mass concentrations of PCNs were very high, with TEQ concentrations exceeding those of PCDD/Fs (Jin et al., 2016; Liu et al., 2016; Zhao et al., 2017a and 2017b). Thus, cement kilns co-processing solid wastes should be given particular consideration for PCN emissions and control.

Although, such diffusive PCN sources also exist in Poland (Orlikowska et al., 2009; Wyrzykowska et al., 2007 and 2009) and elsewhere (Tables 3 and 4), any estimation of the amounts of PCNs from such sources would currently be of dubious quality - mainly because the materials processed in these sources have not been characterised and additionally, the scale of emissions of these sources has not as yet, been studied. Available monitoring data of PCNs emissions was considerably lower than that...
of PCDD/Fs for industrial thermal sources, possible because this is not covered by regulatory requirements and would require dedicated research studies. What is clear is that PCNs are unequivocally formed in thermal processes, the most important of which seem to be waste incineration, cement kilns co-processing with municipal solid waste incineration, hazardous waste incineration, secondary aluminium-, copper-, lead-, magnesium- or zinc smelting plants, iron foundries, iron ore sintering plants and electric arc furnaces and the steelmaking industry. Many of these processes occur in Poland and a national inventory of PCDD/F (and dioxin-like PCBs) emissions from all sources would be a good initial step towards estimating PCN and other emissions in Poland. This would however require considerable effort and financing as well as permission and co-operation from source operators, to undertake the measurements.

The upper bound estimate of total global production has been put at 400,000 metric tons but the amounts (at least, many 10s of tonnes) that are currently emitted unintentionally every year through industrial combustion processes should also be inventoried along with estimates for emissions from bush and forest fires. This would however require considerable national effort, financing and co-operation from source operators.

References
**Introduction:** The details of the POPs formation mechanism on the molecular level are important for controlling POPs formations in industrial thermal processes. Identification of the intermediates, including organic free radicals, during the thermochemical reactions of precursors, such as chlorophenol, is required. In addition, metal compounds play a pivotal role in the catalytic formation of organic pollutants during thermal processes, contributing to critical emissions of organic pollutants such as the infamous dioxins from solid waste incineration processes, secondary metal smelting processes and steelmaking processes, and so on that rich in various metal oxides. Field studies has shown that concentrations and characteristics of organic pollutants generated from different thermal-related industrial processes were quite different. Uncovering the discrepancy of catalysis mechanisms by different metal compounds for organic pollutants formation is instructive for the effective target control of priority organic pollutants from different thermal-related industries.

**Materials and Methods:** The occurrence of free radicals produced during the thermochemical reactions of pentachlorophenol (PCP) as the precursor were monitored by electron paramagnetic resonance (EPR) spectroscopy, which could provide direct evidence for understanding the mechanisms involved in POPs formation. The catalysis pathways of different metal oxides were also proposed on the basis of the organic free radical intermediates identified by EPR and the organic pollutants screened by gas chromatography quadrupole time-of-flight mass spectrometry (GC/Q-TOF-MS).

**Results:** Organic free radicals under the catalysis of different metal oxides were detected by EPR spectrometer. Different organic radical spectrums were acquired when PCP was used as the precursor during the thermal reaction at the temperature range from 298K to 600K under different catalysts, indicating different catalysis mechanisms of different metal compounds. Elucidation of the complex spectrum is the first step to clarify the organic free radical intermediate species. The thermal reaction products of pentachlorophenol under different metal compounds catalysis were identified by GC/Q-TOF-MS. The characteristics of the screened chemicals were quite different on the surface of different catalysts. Chemicals under the catalysis of CuO are of higher chlorination degree, octachlorodibenzo-p-dioxin were only detected in the CuO system with the same condition, indicating that CuO has the strongest catalytic ability to dioxins among the four metal oxides. Under the catalysis of ZnO, chemical of higher oxidation state was dominant, including the benzofenac methyl ester, and some polycyclic aromatic hydrocarbons like 1,4-diisopropynaphthalene. The products from the catalysis of Al2O3 has less chlorine substitution and longer carbon chain, demonstrating that PCP is more prone to dechlorination and alkylation under Al2O3 catalysis.

**Discussion and Conclusion:** We preciously distinguished the multiple organic free radical intermediates during the organic pollutants formation through in-situ detection of electron paramagnetic resonance spectrometry. The differences of organic free radical intermediate species, concentrations and formation mechanisms under the catalysis of different metal compounds were uncovered, which were verified mutually with the characteristics of final organic pollutants screened by time-of-flight mass spectrometry. CuO dominated dehydrogenation reactions of PCP to form pentachlorophenoxy radicals, and the poor stability of organic free radical intermediates on CuO surface made them readily be dimerized to high chlorinated organic pollutants. The specific high proportion of semiquinone radicals and oxygen-containing derivatives in ZnO system indicated that oxidation reactions were predominant. Differently, methyl substituted organic free radical intermediates and long-chain products including the polycyclic aromatic hydrocarbons of high rings were dominant in two polymorphs Al2O3 systems, which demonstrated that Al2O3 has significant advantages for catalyzing alkylation reactions. The consistent characteristics of organic free radical intermediates and final organic pollutants suggested an essential role of free radical intermediates on the organic pollutants formation.

**Acknowledgments:** This work was supported by the National Natural Science Foundation of China (grant numbers 21936007, 21906165, 22076201 and 92143201).

As part of its Green Deal, the European Commission has released in 2022 a "Restrictions Roadmap under the Chemicals Strategy for Sustainability". The aim of the roadmap was to "bring about a toxic-free environment and to protect people and the environment from hazardous chemicals". The roadmap proposes a rolling list with planned restrictions of, groups of substances, among them "Flame Retardants". ECHA has in turn been tasked to develop an overall strategy on flame retardants.

In March 2023, ECHA has published its "Regulatory strategy for Flame Retardants" (FR Strategy), which outlines possible restrictions of both organohalogen- and phosphorous based flame retardants in a grouping approach. In this paper some types of brominated Flame Retardants (BFRs) were identified as a priority for further restrictions.

This presentation will provide an industry perspective of the FR Strategy and the applied grouping approach used by the Strategy. Specifically, the presentation will summarize key points of the FR Strategy regarding BFRs and will discuss the assumptions made in the FR Strategy, which do not always coincide with the current REACH regulation in place. In addition, it will point out the public safety value of flame retardants.
Introduction:
The Flemish Environment Agency (VMM) measures PCDD/Fs, shortly dioxins, and dioxin-like PCBs (DL-PCBs) in deposition to investigate whether these substances are precipitating from the air and whether there is a potential risk of uptake via the food chain. There is currently no Flemish regulation on dioxins and DL-PCBs in deposition. However, the VMM had derived threshold values in 2007 for the sum of 17 dioxins and 12 DL-PCBs in deposition [1]. The current annual average threshold is 8.2 pg TEQWHO1998/(m².day) and was derived using the Tolerable Weekly Intake (TWI) of 14 pg TEQWHO1998/kg bw/week for the group of PCDD/Fs and DL-PCBs proposed by the EU SCF [2]. EFSA revised the TWI for PCDD/Fs and DL-PCBs in 2018. They lowered the TWI to 2 pg TEQWHO2005/kg bw/week [3], which requires a revision of the current Flemish thresholds. This requires the identification of a suitable transfer and exposure model that allows source-to-dose calculations. In addition, a method should be developed to derive threshold values in deposition for the group of dioxins and dioxin-like PCBs.

Materials and Methods:
For source-to-dose modeling, the S-Risk© model can be used, which is used in Flanders to derive soil remediation standards. S-Risk© is a state-of-the-art model for the assessment of exposure and human health risks at contaminated sites. The fate and distribution of chemical contaminants in soil are calculated according to steady-state conservation of mass principles. Deposition in this model is considered as deposition after resuspension of soil instead of as direct deposition from air. However, only deposition data directly from air are available. Consequently, the suitability of S-Risk© to use this data had to be investigated. Physicochemical properties of the 17 dioxins and 12 DL-PCBs were searched for in literature, as well as environmental transfer factors to animal products such as meat, eggs and milk. It was decided to perform the calculations for one congener (2,3,7,8-TCDD) as an indicator for the whole group, since policymakers need a threshold value for the whole group of dioxins and dioxin-like PCBs and 2,3,7,8-TCDD is the most toxic congener. This method was validated by comparison with calculations carried out in 2007 with another model (XtraFood, [1]) and by comparison of predicted concentrations in food with measured concentrations.

Results:
The S-Risk© model could predict fairly well the concentrations in food when compared to measured concentrations. The S-Risk© modeling also results in comparable doses to the ones modeled in 2007, where the deposition from air is explicitly modeled (Figure 1). This confirms the suitability of S-Risk©, if adapted, to model deposition of dioxins and DL-PCBs. Based on the relative contribution of the different considered exposure sources, it was shown that the diffuse background (from commercial food) alone already was enough to exceed the recent TWI of 2 pg TEQWHO2005/kg bw/week in Flanders. This leads to the consideration of different scenarios including scenarios that do not, or only partially, take into account background exposure and focus on different levels of consumption of homegrown food as well as different land use scenarios (agriculture, industry,.....). To illustrate the effect of the different scenarios on the threshold value, several preliminary calculations have been carried out. Sensitivity analyses of the parameters with the highest associated uncertainty (e.g. biotransfer from soil to eggs) have been carried out. These calculations have to be refined later, once the scenarios are approved, and checked for their feasibility with regards to implementation and incorporation into Flemish legislation.

Discussion and Conclusion:
As the diffuse background levels already exceed EFSA’s most recent TWI of 2 pg TEQWHO2005/kg bw/week in Flanders, it will be difficult to derive threshold values specifically for deposition. Therefore, different scenarios and preliminary performed calculations are proposed to policymakers to illustrate the impact of these scenarios on possible threshold values. Furthermore, we performed initial sensitivity analyses on some of the parameters with the highest associated variability. Lastly, we compared the performance of S-Risk© with the XtraFood model from 2007, which yielded satisfying results. Next steps include taking decisions on the final scenarios in consultation with the policymakers, refine the calculations and extend the sensitivity analyses.
Figure 1 Comparison between S-Risk© modeling output (red) and output from the XtraFood model in 2007 (blue, orange and green).

Acknowledgments:
This study is financed by the Flemish Environment Agency.

References:
Human Exposure

P-039  A Fast and Reliable High-throughput Method for Analysis of Hydroxylated Polycyclic Aromatic Hydrocarbons, Cotinine and Creatine in Human Urine by Supercritical Fluid Chromatography Tandem Mass-Spectrometry

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Introduction: Polycyclic aromatic hydrocarbons (PAHs) are occurring naturally in coal, crude oil and gasoline. Humans are exposed to these compounds through several pathways as digestion by eating foods where PAHs have settled from air or consuming grilled/charred meats or foods, by inhalation of motor vehicle exhaust, cigarette and wood smoke or fumes from asphalt roads, and by dermal contact. In the human body the PAHs are metabolized to hydroxy metabolites (OH-PAHs) by cytochrome P450 enzymes in the liver and excreted via the feces and urine. Several PAHs are known to be cancerogenic, but human health effects from exposure to low and moderate concentrations of PAH-mixtures are not well investigated yet. In the present study a novel method for simultaneous analysis of 16 OH-PAHs, cotinine and creatinine in human urine was developed, validated and tested for its applicability within biomonitoring studies.

Materials and Methods: All chemicals and solvents used for sample preparation and instrumental analysis were of LC-MS grade superpure quality. Native and isotope labelled standards were of high purity and purchased from Toronto Research Chemicals, Dr. Ehrenstorfer or Chambridge Isotope Laboratory. Sample material for method development and validation was collected in-house and analysed together with material from the External Quality Assessment Scheme for Organic Substances in Urine, QSEQAS (CTQ The Centre de toxicologie du Quebec, Canada). Sample preparation of 200µL urine was performed in a 96-well format and by a liquid handler equipped with an 8-channel pipetting arm, a robotic manipulator arm, a solid-phase extraction station and a shaker (Tecan Freedom Evo 200, Männedorf, Switzerland). After an enzymatic deconjugation treatment of the samples, a solid-phase extraction on a reversed phase stationary material was conducted for simultaneous extraction of OH-PAHs, cotinine and creatinine. Instrumental analysis was performed on an ultra-performance convergence chromatograph coupled to a tandem mass-spectrometer (Acquity UPC2-Xevo TQ-XS, Waters, Milford, MA, USA).

Results: Chromatographic baseline separation was achieved for all 16 OH-PAH analytes, cotinine and creatinine. To our knowledge this is demonstrated for the first time. Results from the validation work together with examples of real samples demonstrate the applicability of the developed method for biomonitoring and exposure studies.

Discussion and Conclusion: Previous published methods relayed on liquid chromatography for separation of the OH-PAHs. Baseline separation of some OH-PAH isomers (e.g. 1-hydroxyphenanthrene and 9-hydroxyphenanthrene or 2-hydroxyphenanthrene and 3-hydroxyphenanthrene, could not be achieved (Shang Ting et al., 2019), which makes a specific identification and quantification of these individual analytes impossible. Further, our method provides a simultaneous extraction and analysis of cotinine and creatinine together with OH-PAHs, which is extremely important for population and biobank studies that have limited sample volume stored. Our developed method exhibits novel opportunities for investigation of OH-PAH concentrations in different research cohorts related to associations between OH-PAHs and different clinical biochemical variables together with medical parameters for a better understanding of adverse health outcomes and possible causal relationships.

Acknowledgments: The authors are gratefully for the financial support of the Northern Norway Regional Health Authority (Helse Nord RHF) and the Department of Laboratory Medicine, University Hospital of North-Norway. We thank also UiT The Arctic University of Norway for funding of the Acquity UPC2-Xevo TQ-XS system through the interdisciplinary strategic project High North Population Studies.

1. Introduction:
Polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins/dibenzofurans (PCDDs/PCDFs), dichlorodiphenyltrichloroethane-ethane (p,p’-DDT) and its metabolite p,p’-DDE were monitored in breast milk. These toxic organic pollutants have various adverse effects on human health (e.g. endocrine disruption, immune dysfunction, neurological, dental and birth defects, cancer, etc., Langer et al., 2008).

They are extremely resistant to environmental decomposition. After entering the human body, they accumulate in fat. Therefore, breast milk represents a potential risk for child’s development and health (Hernández et al., 2020, Malgorzata Ulaszewska et al., 2011). Environmental pollutants are exceptionally dangerous because they damage not only immune and endocrine system of infants and adults but also the developing fetus in maternal body.

Within our study, nursing mothers were from all over Slovakia. We focused not only on sites with chemical contamination but we monitored a wide range of locations during 30 years.

Decreasing tendencies of contaminant concentration were recorded. Samples were collected from 1992 to 2020 during various projects and studies. A total of 489 samples were analysed over 30 years but PCB levels in the general population in Slovakia are still rather high because PCBs were manufactured in eastern Slovakia in Chemko Co., Strážske, until 1984 (Kočan et al., 1995, Kočan et al., 1999, Chovancová et al., 2011).

2. Material and Methods:

Extraction
The starting point in each study was the selection of sampling regions, searching and asking mothers for milk samples. Primipara mothers were without pregnancy and parturition complications. Samples were collected into clean glass bottles with teflon seals.

All samples were kept frozen at -20 oC until analytical process. 50–100 ml thawed individual breast milk samples were taken for analysis. Milk fat (3–5 g), extracted by liquid-liquid extraction (Petrik et al., 2001), was dissolved in n-hexane and spiked with a mixture of labelled compounds. After removal of fat on a high-capacity acid-silica gel column, PCDDs/Fs, dioxin-like PCBs (DL-PCBs) were separated on a Power-PrepTM semi-automated clean-up system (Fluid Management Systems, Waltham, MA, USA) with pre-packed multi-layer silica, basic alumina and carbon columns. 10 µl of n-nonane as a keeper was added to each eluate prior the evaporation.

Chemical Analyses
In 1992, analyses were performed on HP5890 gas chromatograph equipped with 63Ni ECD. From 2000 to present, analyses of 2,3,7,8-substituted PCDD/PCDFs, PCBs and DDE/DDT were performed using HP 6890 GC (Hewlett-Packard, Palo Alto, CA, USA) coupled to an MAT95 XP (ThermoFinnigan, Bremen, Germany), operating at a resolution of 10000 in selected ion monitoring mode. PCDD/F, DL-PCB congeners and DDT/DDE were separated on DB-5 and/or DB-5MS column (60 m, 0.25 mm I.D., 0.25 µm film thickness).

Quantification was carried out by isotope dilution methods US EPA 1613 and 1668. Prior to mass spectral analysis, recovery standards of labelled 1,2,3,4-TCDD and 1,2,3,7,8,9-HxCDD were added to the fractions containing PCDDs/PCDFs and non-ortho PCBs, and recovery standards of labelled PCBs were added to the fractions containing of PCBs.

QA/QC
Each batch of 10 samples included one method blank. To verify the accuracy of the analytical process, a QC sample (certified reference material) was used. All measurements were carried out in an accredited laboratory (ISO/IEC 17 025).

3. Results and Discussion:
General characteristics of the mothers were reported in relevant studies (age, BMI, birth weight of children, smoking and eating habits). Breast milk is a unique matrix for biomonitoring of environmental contaminants stored in body fat (Klinčić et al., 2016, Rawn et al., 2017, Hernández et al. 2020). All donor mothers belonged to general human population (no professional exposure to contamination). The Levels of PCDDs, PCDFs and DL-PCBs are expressed as toxic equivalent (TEQ) calculated using toxic equivalency factors TEF 2005.

First comprehensive study was done between 1992–1994 (n=50), then 2000 (n=60), 2006 (n=51), 2014 (n=298) and the last in 2020 (n=50). For each individual study, average values were calculated from the measured results. The concentrations of PCDD/F, PCB congeners (also expressed as TEQ values) and DDT/DDE in breast milk have a decreasing tendency. Sum of PCDDs, PCDFs and DL-PCBs as TEQ values are shown in the Figure 1.
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Figure 1: Sum of PCDDs, PCDFs and dl-PCBs as TEQ values (pg/g fat) in breast milk (y. 1992-1994, 2000, 2006, 2020)

After thirty years of monitoring, the values have decreased to a quarter of the value recorded in 1992. The decrease of contamination can best be seen in the concentration of IND-PCBs (PCB-28, -52, -101, -138, -153, -180) in breast milk (Figure 2). Figure 3 shows the decrease in the DDT+DDE concentration over the time.


Figure 3: Sum of DDT and DDE (ng/g fat) in breast milk (y. 1992-1994, 2006, 2014, 2020)

Mothers in the first study (1992–1994) were born in 1966–75, i.e. they spent approximately half of their lives in the period of large-scale PCB production in Eastern Slovakia (Chemko Strážske, until 1984). In the 1970’s the use of DDT was banned and concentrations of DDT and DDE dropped rapidly. However, DDT was still present in the environment in immense amounts, as evidenced by the concentration of DDT+DDE in breast milk in the first study.
4. Conclusion:
Intake of PCBs, PCDDs/PCDFs and DDT+DDE by the infant in the first weeks of breastfeeding, when breast milk is the only source of nutrition, should be monitored. In the past, mothers were exposed to a contaminated environment which led to high levels of contaminants in human milk. High contamination with POPs in Slovakia is due to industrial and agricultural activities in the past, and therefore should be further monitored. In spite of that, the benefits of the breast-feeding outweigh the risks, especially after such a significant decrease of contamination.

5. Acknowledgments:
This research was supported by projects:

- Chlorinated aromatic compounds in the human organism from selected model areas of the Slovak Republic., Project No. 93-535-03-32, (1992-1994)
- Early life exposure to risk factors for obesity and development in 3-year-old children. OBEZOGEN, APVV-0444-11 (2011-2016)

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Human Exposure

P-041 Recalculation of dietary exposure to PCDD/Fs and dl-PCBs in China by TEF2022

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Introduction: Polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) and dioxin-like polychlorinated biphenyls (dl-PCBs) are typical persistent organic compounds (POPs). For non-occupational population, exposure to PCDD/Fs and dl-PCBs is mainly through the diet, with foods of animal origin being the most source. Because of their persistence, high toxicity and ubiquity in foods, there is still concern for dietary intake that could pose potential risk on human health. Thus, we conducted the 6th China Total Diet Study (CTDS) during the period of 2016-2020 and dietary exposure of Chinese adults to PCDD/Fs and dl-PCBs was assessed by the 2005 World Health Organization (WHO) Toxic Equivalency Factors (TEF) scheme. In October 2022, WHO convened an expert panel to reevaluated the TEF that released as TEF 2022. In this study, we applied these new TEFs to re-calculate the total amount of toxic equivalence (TEQ) for various foods and re-evaluate the dietary exposure to these chemicals of Chinese adults.

Materials and Methods: 96 composites of animal origin foods, including aquatic foods, meat and meat products, egg and egg products, and milk and dairy products, were selected from the 6th CTDS including 24 provinces of China. 17 congeners of 2,3,7,8-substituted PCDD/Fs and 12 congeners of dl-PCBs designated by WHO were determined by a high resolution gas chromatograph–high resolution mass spectrometer (HRGC–HRMS, DFS, ThermoScientific, Germany). TEF 2005 and TEF2022 was applied to calculate the total amount of TEQ. When an amount was under the detection limit (LOD), the value was assumed to be equal to LOD as upper bound estimation (UB). Dietary intake was calculated by multiplying the amount of TEQ (pg TEQ g⁻¹, fresh weight) by the consumption data (g day⁻¹ kg⁻¹ body weight).

Results: When TEF 2005 was applied, the total amount of TEQ (mean ± standard deviation) was 0.14 ± 0.07 pg TEQ g⁻¹ in aquatic foods, 0.08 ± 0.04 pg TEQ g⁻¹ in meats and meat products, 0.06 ± 0.04 pg TEQ g⁻¹ in eggs and egg products, and 0.03 ± 0.02 pg TEQ g⁻¹ in milk and dairy products, respectively. The dietary intake was 1.64 ± 1.11 pg TEQ kg⁻¹ bw week⁻¹ with a range of 0.31 to 4.46 pg TEQ kg⁻¹ bw week⁻¹.

When TEF 2022 was applied, the total amount of TEQ was 0.10 ± 0.05 pg TEQ g⁻¹ in aquatic foods, 0.08 ± 0.05 pg TEQ g⁻¹ in meats and meat products, 0.05 ± 0.03 pg TEQ g⁻¹ in eggs and egg products, and 0.02 ± 0.01 pg TEQ g⁻¹ in milk and dairy products, respectively. The dietary intake was 1.31 ± 0.75 pg TEQ kg⁻¹ bw week⁻¹ with a range of 0.31 to 3.18 pg TEQ kg⁻¹ bw week⁻¹.

Discussion and Conclusion: When applying the new TEF scheme, the total amount of TEQ tend to be lower. By comparison with the application of TEF 2005, the mean levels of total TEQ in aquatic foods reduced by 29.9%, 9.7% in meats and meat products, 13.2% in eggs and egg products, and 31.2% in milk and dairy products, respectively. However, statistical difference was only observed in the amount of total TEQ in aquatic foods (p=0.02, t-test). In addition, the mean of dietary intake reduced by 20.1%, but there was no statistical difference (p>0.05). The contributions of various congeners of PCDD/Fs and dl-PCBs to the total amount of TEQ changed greatly, especially for PCB126 and 2,3,4,7,8-PeCDF, showing in Fig.1. In conclusion, applying the new TEFs tend to low the total amount of TEQ in food samples as well as dietary exposure and alter the profiles of congeners contribution to the total amount of TEQ.

Fig.1 The average contribution of congeners of PCDD/Fs and dl-PCBs to total amount of TEQ

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P-042  Intergenerational chemical risk assessment in humans using epigenetic epidemiology studies and machine learning approaches

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Introduction:

According to multiple animal studies the germ line epigenome is sensitive to various factors, including chronic exposure to harmful lifestyle factors and environmental pollutants 1. If these non-genetic alterations in germ cells can be inherited from parent-to-child in humans, it is important to improve our understanding of environment-epigenome interactions.

In a pilot study, we evaluated a few imprinted genes and found that men with higher concentrations of urinary metabolites of organophosphate ester (OPE) flame retardants had higher fractions of sperm that were aberrantly methylated, compared to men with low concentrations of OPEs 2. Notably, usage of OPEs is increasing in consumer products and construction materials after a ban on brominated flame retardants 3. Our early results contributed to the hypothesis that chronic or early environmental exposures can change the human epigenome through the male germ line. This concept, referred to as the Paternal Origin of Health and Disease (POHaD) theory, was proposed about 7 years ago and is still an underdeveloped view on how humans may adapt (or respond) to environmental changes 4, 5, 6, 7.

Numerous animal studies have shown supporting evidence that exposure to toxicants can induce sperm epimutations that have long-term phenotypic effects, up to the third generation 8, 9. However, alternatives to animal testing are needed and current in vitro tests cannot provide an answer to questions about epigenetic inheritance in humans. On the other hand, the paternal contribution of early exposures to man-made chemicals is often overlooked in traditional epidemiological studies.

Here we propose a proof-of-concept method where existing human studies could be used, including comprehensive datasets and samples, combined with machine learning approaches. This concept could serve the Partnership for the Assessment of Risks from Chemicals (PARC) initiative. PARC is a research and innovation program funded by Horizon Europe, bringing together over 200 partners to support exposure modeling, human biomonitoring, next-generation chemical risk assessment and risk management incorporating both human health and the environment in a “One Health” approach. Through the combination of well-designed human observational studies and advanced machine learning based approaches, new theories could be developed about adverse biological effects from chemical exposures, prior to further validation studies or in silico simulation tests.

Materials and Methods:

We selected existing epidemiological studies to assess epigenetic effects in parents and/or children in response to earlier exposures to environmental pollutants. Selected datasets included individual information of subjects and at least two of the following requirements: 1. measured metabolites of environmental chemicals to which populations were exposed to on a daily basis, such as endocrine disruptors (EDCs) or OPEs, e.g., used as flame retardants or plasticizers; 2. measured epigenetic factors
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in human specimens that could be linked to inheritance from one generation to the next (e.g., male germ cells and/or in children); 3. diseases outcomes or risk assessment in children. Ethical approvals for these studies have been obtained.

OPEs have been detected in human matrices via liquid chromatography tandem mass-spectrometry. The following metabolites of OPEs were assessed: bis(1,3-dichloro-2-propyl) phosphate (BDCIPP), bis(1-chloro-2-propyl) phosphate (BCIPP), diphenyl phosphate (DPHP), isoproplyphenyl phenyl phosphate (ipPDPP), terbutlyphenyl phenyl phosphate (tbPDPP), and Tris(2-chloroethyl) phosphate (TCEP).

Genome-wide epigenetic measurements were mainly obtained through the Infinium HumanMethylation450 array (450K array). This technique uses bisulfite treatment to convert unmethylated cytosines, but not methylated cytosines, to uracils, generating a C/T polymorphism at CpG sites after DNA amplification; this is readily measurable with the Infinium technology. We performed data preprocessing as described earlier 10, including sample quality control, filtering of probes with low intensities, and normalization (Subset-quantile Within Array Normalization (SWAN) versus Beta Mixture Quantile (BMQ) Normalization).

Both supervised and unsupervised machine learning (ML) algorithms will be implemented for data integration. Available data from the arrays, associated meta data, and chemical information will be integrated using a naïve vertical integration after standardization to generate a single unified dataset. ML models, that try to identify patterns within the data, will be used to identify key multivariate components leading to the observed adverse outcome or to the chemical concentration highlighting the methylation sites affected by these chemicals. Network based approaches may further allow us to identify potential combinations of genes and metadata that associate more strongly with specific outcomes leading to new knowledge on longer-term effects of the exposure of interest. Probabilistic graphical modeling, such as a Bayesian network, will be explored and could be beneficial, opening new opportunities to link a specific exposure to toxicological effects in human.

Results:

The use of carefully selected existing human datasets can serve as a starting point to generate new epigenetic biomarkers. These novel effect biomarkers could help develop the next-generation risk assessment tools or increase our understanding of adverse outcome pathways (AOPs) or networks of AOPs. To build our concept, we here describe protocols and designs of three epidemiological studies, including cross-sectional studies and prospective cohorts.

First, The Influence of the Environment on Gametic Epigenetic Reprogramming (TIEGER) study included nearly 90 young male volunteers from North Carolina, U.S.A., recruited for a one-time measurement of OPEs metabolites (TDCIPP, TCIPP, TPHP, ipPDPP and tbPDPP) in urine. We found that most men (>90%) had been exposed to at least 3 of these OPEs. DNA methylation was measured in sperm at 485,512 CpGs using the 450K array. These results have been preprocessed as described in our methods section. Additional health-related determinants such as lifestyle, social environment, medical condition, etc. have also been included. Hence, the TIEGER data are ready for exposure-outcome correlation analyses and accurate selection of differentially DNA methylated genes by the exposure of interest.

Second, The Epigenetic Legacy of Paternal Obesity (ELPO) cohort includes information about fathers, mothers, embryos, and newborns. Data and samples were obtained from 100 parents at the Leuven University Fertility Center, Belgium. This dataset includes anthropometric characteristics, socio-demographic information, lifestyle, and dietary records. Blood, urine, and sperm samples were collected for measurement of epigenetic outcomes and chemical exposures, offering the opportunity to identify epigenetic signatures that persist from one generation to the next, in the same families.

Third, The Genetics, Early Life Environmental Exposures and Infant Development in Andalucía (GENEIDA) study is a Spanish comprehensive birth cohort where metabolites of OPEs (TDCIPP, TCIPP and TCEP) have been quantified in 529 parent-child pairs. In addition to extensively collected data on nutritional and lifestyle-related factors of the parents, neurodevelopmental assessments were performed in the children at the age of 12 and 24 months; additionally, a physiologically-based pharmacokinetic model (PBPK) has been developed. This dataset could be used for biomarker selection and future screening for neurodevelopmental disorders originating from earlier exposures to OPEs.

Briefly, ML approaches will resolve potential imbalance issues frequently occurring in epigenetic datasets and allow efficient feature selection 12. Finally, using ML data modeling long-term effects of chemical exposures will be delineated.

Discussion:

Studying intergenerational health effects of chemical exposures in humans is challenging. A holistic approach including knowledge of epigenetic mechanisms of inheritance, carefully selected samples, genome-wide analyses, lifestyle-related data of human volunteers in their natural circumstances (also including exposure to mixtures of chemicals) and advanced computational modeling may bring advantage of less biased new findings, generated from observational studies. We foresee that the use of available human data from epidemiological studies (e.g., before, during and after pregnancy), combined with open access data
of the human epigenome and transcriptome, machine learning methods and appropriate data integration techniques (e.g., multiomics), will allow novel opportunities in intergenerational chemical risk assessment. We believe this proposed approach is a first step in the yet unexplored territory of chemical assessment and/or toxicity testing.

Conclusion:
In the context of public health, a major concern is that intergenerational epigenetic effects from environmental exposures are generally not considered in chemical risk assessment, due to data limits within single studies and lack of prospectively collected human samples from multiple generations. For obvious reasons some of these limitations are inevitable in human studies. While it is well established that a woman’s lifestyle and environmental exposures before and during pregnancy can interfere with an offspring’s health, preconceptional care and exposures in men are generally given less consideration. However, just like women, men carry epigenetic signatures related to their preconceptional conditions or exposures. Next to daily exposures from pollution, some occupational exposures in men before conception may also affect the male germ line, and subsequently the health of offspring. We believe there is an urgent need to increase the awareness of the role of both parents; hence, large epidemiological studies, such as birth cohorts and occupational studies, should also include paternal exposures in their study design. Finally, integrating multiple datasets and implementation of novel ML methods could help fill the gaps inherent to human data sets.

Acknowledgments:
Health and the Environment Award (TIEGER study) from Duke University School of Medicine and Duke Nicholas School of the Environment, FWO grant G0C8523N, co-funded by the FPS Health, Food Chain Safety and Environment, Belgium. Institute of Health Carlos III (grants: P118/01156; P117/00638; P113/01559), including the European Regional Development Fund (FEDER). Regional Health Council of Andalusia (Spain) (grants: PI-0243-2019; PI-0508-2016; PI-0205-2016). Additionally, this work was carried out in the framework of the co-funded European Partnership for the Assessment of Risks from Chemicals (PARC) and has received funding from the European Union’s Horizon Europe research and innovation program under Grant Agreement No 101057014. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or the Health and Digital Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.

References:
Human Exposure

P-043 Levels of PCDD/Fs and dl-PCBs in human serum of adult Spanish population by GC-HRMS

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Introduction: The global issue of human exposure to polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and dioxin-like polychlorinated biphenyls (dl-PCBs) arises from their unintentional production. These substances, known as toxic bioaccumulative organic compounds, have been categorized as persistent organic pollutants (POPs) under the Stockholm Convention [1]. A total of 17 PCDD/Fs and 12 dl-PCBs have been identified as harmful to human health, exhibiting a wide range of toxic effects [2]. 100 human serum samples of adult population from the Valencia Region of Spain have been analyzed in this work.

Materials and Methods: The methodology to analyse the 17 PCDD/Fs and 12 dl-PCBs includes extraction by selective pressurized liquid extraction (SPLE) followed by a clean-up multicolumn step. Samples were injected by gas chromatography coupled to high-resolution mass spectrometry (GC-HRMS).

Results: For PCDD/Fs, the congeners with the highest detection frequency in the analyzed samples were OCDD (detected in 100% of samples) and 1,2,3,4,6,7,8-HpCDD (85% frequency of detection). For dl-PCBs, PCB-118, PCB-105 and PCB-156 were the congeners highest detected, being the only three observed in all samples. The total concentrations of the sum of PCDD, PCDF, and dl-PCBs in the analyzed samples (considering upper-bound) ranged from 2.80 to 22.30 pg TEQ/g lipid, with an estimated geometric mean (GM) of 7.69 pg TEQ/g lipid.

Discussion and Conclusion: The present study shows PCDD/Fs and dl-PCBs concentrations in serum samples of Spanish adult population living in the Valencia Region. The obtained levels are similar to other human biomonitoring studies in Spain [3]. Further studies focused on human exposure will be necessary to assess the risk of population due to the exposition to these pollutants.

Acknowledgments: This work was developed in the framework of the BIOMOVAL project with the support of the Public Health Directorate of Valencia and FISABIO. The study was co-funded by the European Union through the European Regional Development Fund Operational Programme (ERDF) of the Valencia Region (2014–2020).

References:
Human Exposure

P-046 A review of the human exposure to persistent and mobile chemicals and their potential health risk assessments

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Introduction: Persistent and mobile chemicals (PMs) are highly polar organic chemicals of anthropogenic origin, which have been documented as an emerging issue of concern for environmental and human health. However, the comprehensive information regarding the human exposure to PMs is limited. The aim of this work was to review the recent knowledge on human exposure to PMs and to assess potential health risks based on the relevant published reference data.

Materials and Methods: Eight groups of PMs: melamine (MEL), quaternary ammonium compounds (QACs), benzotriazoles (BTRs), benzothiazoles (BTHs), 1,4-dioxane (1,4-D), 1,3-di-o-tolyguanidine (DTG), 1,3-diphenylguanidine (DPG), and trifluoromethane sulfonic acid (TFMS), including their derivatives, were selected based on the persistency and mobility criteria provided by the German Environment Agency (UBA). As a result, 28 PM chemicals were included in this review. Exposure levels of each group of PM were retrieved for several human biomatrices, such as urine, blood, and breast milk. The values of estimated daily intakes (EDIs) from both internal and external human exposure, through e.g., drinking water, indoor dust, food and consumer products, were compared to relevant toxicity reference data represented by tolerable daily intake (TDI), acceptable daily intake (ADI) or reference dose (RfD) values.

Results: The reviewed data mostly included general adult population, followed by pregnant women. Urine was the most used matrix for human biomonitoring of MELs, BTRs, and BTHs, while blood was the most common matrix for QACs, 1,4-D, DPG, and DTG. Among the PMs selected in this review, higher concentrations of MELs and BTH were observed in urine. Furthermore, MELs was detected in all urine samples (DF=100%). Blood levels of QACs were significantly higher in samples collected before vs. those during the COVID-19 pandemic. MELs and QACs were detected also in breast milk, whereby 10 fold higher concentration were observed for MELs compared to QACs. Both MELs and QACs were higher in urine than in breast milk. Currently, very limited biomonitoring studies on DPG and DTG were available, moreover, no reports regarding TFMS were available.

Discussion and Conclusion: None of the reviewed PMs showed EDIs exceeding the current TDI or RfD values. This indicates that internal and external exposure levels of these PMs and in the studied general populations are less probable to lead to health effects. However, exposure levels are unknown for many other PMs and in vulnerable populations. Further comprehensive biomonitoring studies including vulnerable populations and state-of-the-art analytical methods are needed.
P-047 20 Years Later: PBDEs in fish from U.S. sites with historically extreme contamination

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Introduction: Polybrominated diphenyl ethers (PBDEs) are flame-retardants historically added to polymers to prevent or slow the growth of fire for an array of polymeric consumer products (e.g. foam padding, furniture, textiles, electronics). As a result of their broad usage, tendency to migrate from products and the propensity of these to degrade into readily transportable micro- and nano-plastics, PBDEs have become ubiquitous environmental contaminants. Due to their dissemination, environmental and human health concerns PBDEs were added to Stockholm Convention Annex A, restricting their use globally. Shortly thereafter, environmental levels retreated from their peak at the start of this century. However, the rate of decrease now appears to be lessening, e.g., as concentrations in predatory fish of North America’s Great Lakes remaining constant since 2011.2 Similar trends were also observed in dolphins from the Mediterranean Sea where concentrations were nearly halved between 1990 and 2004/2009 but remained relatively stable through 2018.3 These observations may be inherent due to PBDEs’ global dissemination and their ability to persist and biomagnify once released into the environment. However, “new” sources may also be contributing, e.g. from PBDE-containing durable goods still in use, releases during recycling, the entry of contaminated recycled products into commerce and special-use authorizations.4 Therefore, to further evaluate environmental PBDE trends this study re-examined a U.S. freshwater river system reported to have the highest PBDE fish tissue burdens in the world.5 By analyzing archived fish tissue samples, contemporary (2018 – 2020) levels were compared to historic 1999-2000 and 2007 levels of the Dan and Roanoke R. (Virginia). Results provided useful data on environmental trends of PBDEs required to update assessments of human and environmental health risks. The exploitation of archived tissue samples also provided a sustainable and substantial cost savings approach over collection of new material.

Materials and Method: Samples were obtained from the VIMS fish tissue (fillets) archive, originally collected during Virginia’s Department of Environmental Quality annual PCB fish tissue survey. From these, 76 tissue composites were selected for PBDE analysis. These were collected between 2018 - 2020 from 16 sites along Roanoke, Dan and Hyco Rivers, representing 270 river km. Purified sample extracts were analyzed for PBDEs by atmospheric pressure gas chromatography/quadrupole time-of-flight Mass Spectrometry (GC/QTOF-MS).

Results: PBDEs were detected in fish at all 16 collection sites and in 93% of the composites. Concentrations (∑7PBDE) ranged from nd to 16,300 ng g-1 lipid wet (lw). The highest PBDE level was observed in a catfish collected on the Dan R., downstream from its confluence with the Hyco R. The world’s highest PBDE fish level was previously reported (1999) near the mouth of the Hyco.5 PBDE concentrations along the Roanoke R. have dropped by >75% over the past 20-years. Reduction rates between collection sites were similar, with no individual site indicating greater reduction over the next, 7.1% RPD. Declining concentration trends (>92%) were also observed along the upper Dan R. to its confluence with the Hyco R. thereafter concentrations spiked 10-fold. Levels within the Hyco R. have declined through 2007 at an estimated annual rate of 30%, but reductions during the past 13-years have diminished to only a 1.2% rate. The limited data collected suggest that levels on the Dan R. downstream from the Hyco R. have actually increased at an estimated 8% annual rate since 2007.

Discussion and Conclusion: Over 20 years after our initial study, and over a decade after PBDEs were restricted from commerce, PBDE contamination of fish in the Dan and Roanoke rivers remain. The current average Dan R. tissue concentration is 3-times higher than that of the Roanoke R., indicating that the Hyco R. remains a major contributor of PBDEs to the Dan R. The average PBDE levels of Dan and Roanoke R. fish exceed maxima observed in riverine systems of Europe and Asia.6 They were up 1000’s of times higher than the environmental quality standard (EQS) set by European Parliament for aquatic biota (at 0.0085 ng g-1 weight wet).6 Within North America, levels we observed in piscivorous fish species of the Dan and Roanoke R. were twice those of North America’s Great Lakes1 and exceed by 10-fold those observed in carp across Illinois, USA.7 Therefore, to protect the environment and human health environmental monitoring of PBDEs should remain a priority for regulatory agencies with the goal to identify and eliminate PBDE sources.

References:
Legacy and Emerging Flame Retardants: Occurrence and Exposure

P-048 Human exposure to short-chain chlorinated paraffins and organophosphate flame retardants in relation to paired multiple sources

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Introduction: Short chain chlorinated paraffins (SCCPs) and Organophosphate flame retardants (OPFRs) are emerging flame retardants manufactured to replace the so-called legacy flame retardants (Ekpe et al., 2020) and some are classified as emerging halogenated flame retardants. Most of the emerging halogenated flame retardants are additive in nature (i.e. added or mixed to their constituted materials, rather than being chemically bonded to them). Notably, following the reports of previous studies, the potential human exposure pathways of SCCPs and OPFRs include inhalation, ingestion, and dermal sorption (Kim et al., 2019), however, in relation to the body burdens of these emerging flame retardants, the aforementioned exposure pathways have been extensively studied for single matrix sources, such as dust, air, or diet (Yuan et al., 2021). Furthermore, the correlations between multiple sample types with respect to human exposure have also not been reported. Therefore, in this present study, the levels, and distributions of SCCPs and 13 OPFRs were determined in human serum, hair, and paired multiple exposure sources, including one-day composite food, drinking water, and house dust. In addition, the correlations between human serum/hair and multiple exposure sources were assessed to investigate their possible exposure routes. Finally, daily intakes and contribution of SCCPs and OPFRs through various ingestion pathways (food/drinking water consumption and dust ingestion) were determined.

Materials and Methods: Samples of human serum, hair, and paired multiple exposure sources (one-day composite food, drinking water, and house dust) were collected from each of the 50 participants (19 children and 31 adults from 13 families) between April and October 2014 in the Seoul metropolitan area of South Korea. The participants comprised of 27 males and 23 females, and their ages ranged from 10–88 years old. One-day composite food samples (i.e., typical Korean diet, including boiled rice, kimchi, meat, and various seafood) were collected from each of the 50 participants. Thirteen drinking water each (boiled, bottled, filtered, and tea) and house dust samples were collected from each of the 13 houses (families) where the 50 participants were living.

Results: The levels of SCCPs in serum of adults were significantly higher than those of juvenile, while there was no statistical difference with gender (Mann-Whitney U test, \( p < 0.05 \)). In addition, significant correlations in OPFRs levels were observed between serum and drinking water, as well as hair and food using Spearman correlation, and we strictly confirmed this phenomenon from MLR result, and food consumption was one of the important human exposure pathways to SCCPs. There was no obvious risk posed from OPFRs daily intake.

Discussion and Conclusion: To the best of our knowledge, this study is the first to evaluate the human exposure to parent OPFRs in relation to multiple exposure routes based on data obtained from same individual sample groups. In the case of SCCPs, although a recent previous study had evaluated the major exposure of SCCPs in serum via multiple routes in a cohort study (Yuan et al., 2022), present study is the first to consider the exposure dynamics of SCCPs in hair via multiple routes based on samples obtained from same individuals.

Acknowledgments: This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by Ministry of Education (NRF-2021R1A2C2006517, NRF-2021R1A6A1A03039572)

References:
Introduction:
Persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) have been widely detected in birds worldwide (Chen and Hale, 2010). However, the bioaccumulation patterns are highly variable among different bird species due to the multiple feed and preferential biomagnification of certain POPs in food chains (Chen and Hale, 2010). As a promising tool, quantitative fatty acid signature analysis (QFASA) could quantify food source of predators and help to obtain reliable biomagnification factors (BMFs) of contaminants in food chains.

The common kingfisher (Alcedo atthis) is a widely distributed piscivorous bird species that serves as an environmental indicator. Previous studies have reported the bioaccumulation patterns of POPs in kingfishers. However, the sources and biomagnification processes of different POPs in kingfishers remain unclear. Elucidating these issues can provide valuable insights that may inform regulatory policies and management strategies aimed at reducing the use of POPs and protecting human health.

Materials and Methods:
The present study investigated 28 PCBs, 15 PBDEs and 19 fatty acids in 27 kingfishers and 8 prey species from the wetlands of South China. Internal standards included CBs 24, 82, and 198; BDEs 118, 128, and 13C-BDE 209. Recovery standards included CBs 30, 65, and 204; BDEs 77, 181, and 205. All standards were purchased from Accustandard Inc. (USA). QFASA was conducted using RStudio 1.4.1717.

Results:
Kingfishers occupy the highest trophic level, while the 13C values of samples indicate that all species are potential diet for kingfishers. 16:0, 18:0 and 18:1n-9 were the main fatty acids in all samples. Median PCB and PBDE concentrations in kingfishers were 32500 and 1230 ng/g lw, respectively, compared to a range of 3690−42800 ng/g lw and 452−3420 ng/g lw in prey species. In this study, the main PBDE congeners were BDEs 47, 153, 154, and 209, while CBs 118, 138, and 153 dominated the PCBs. The BMFs and TMFs of POPs ranged from 0.05 to 9.86 and 0.8 to 5.61, respectively, and showed a parabolic relationship with log $K_{OW}$, peaking at 7.

Discussion and Conclusion:
The concentrations of PCBs in this study were higher than those found in passerine bird species from the Pearl River Delta but lower than osprey eggs collected from Chesapeake Bay. The concentrations of PBDEs in this study were higher than those found in birds from developed countries, but lower than those found in gulls in Canada. The dominance of CB 118, 128 and 153 in PCBs was consistent with previous studies on birds. However, the compositions of PBDEs in this study differ from that reported for other waterbird taxa such as terns and falcons (where BDE 47 is the major congener).

QFASA results showed that metiza lineata and common carp were the predominant diets, with contributions of 48.7% and 46.3% to total diet of kingfishers. Further calculations of daily exposure doses for kingfishers from different prey species revealed that pelagic- and benthic- prey species were the primary sources of less- and more-hydrophobic contaminants for kingfishers. The log-transformed BMFs and TMFs showed a significant negative correlation with the whole-body elimination rates of POPs in kingfishers ($p<0.01$), which indicates that elimination capacity strongly influenced the biomagnification of POPs in kingfishers.

Acknowledgments: This study was financially supported by the National Natural Science Foundation of China (Nos. 41931290, 42177205 and 42277242).

References:
Today's lifestyle, predominantly bound to interior spaces where people spend approximately 90% of their time, affects increased exposure to the indoor pollutants, including polybrominated diphenyl ethers (PBDEs). PBDEs accumulate in house dust, the ingestion of which is the most important route of exposure for humans besides diet. The exception are breastfed infants, the most sensitive population group, for whom breast milk is the dominant exposure pathway to PBDEs. Since all lipophilic pollutants, including PBDEs, tend to bioaccumulate in breast milk, it is a commonly used matrix for biomonitoring, and the advantage is that it provides information about the mother/adult and infant exposure to PBDEs. In this part of Europe, there is a knowledge gap on PBDE levels in human samples, and in general only a few papers have analyzed house dust and breast milk as matched samples. With this in mind, we conducted an integrated study on the levels of PBDE congeners in house dust and human milk samples from women (N = 30) living in these households in Zagreb. Based on the obtained data, possible correlations of the PBDEs detected in the two analyzed matrices were assessed.

Seven PBDE congeners (BDE-28, -47, -99, -100, -153, -154, and -183) were extracted from both matrices by microwave-assisted extraction (MAE) with n-hexane:acetone (1:1, v/v) as solvent mixture. The clean-up procedure using an in-lab prepared multilayer silica column and n-hexane:dichloromethane (4:1, v/v) as elution solvent was used for dust and milk extract purification, while in the case of milk extracts it was necessary to first remove the lipids with concentrated sulfuric acid. Analysis of the purified samples was performed on a dual column gas chromatograph with micro electron capture detectors.

The sum of the mass fractions of detected PBDE congeners (∑PBDEs) in house dust samples ranged from 0.24 to 523 ng g⁻¹ dust in positive samples, with BDE-99 and BDE-47 being the most abundant congeners, followed by BDE-183. Lower ∑PBDEs were observed in human milk, ranging from 0.16 to 6.30 ng g⁻¹ lipid weight. BDE-153 was the only congener detected in all human milk samples, being the most abundant congener in 50% of samples, and BDE-99 in 23% of samples.

Statistically significant correlations were found between BDE-153 in breast milk and congeners BDE-47, BDE-99, BDE-100 detected in house dust. This indicated that mothers were most exposed to the “penta” formulation in their households, which was mainly used as an additive in polyurethane foam and textiles. Our results are consistent with those obtained in previous studies, which also found certain positive associations between PBDE concentrations in human milk and dust [1,2]. The analysis of BDE-209, mostly used in electronic equipment, as well as food analysis to cover the second most important PBDE intake route, would advance this work further.

This work has been supported in part by the Croatian Science Foundation under the project HrZZ-UIP-2017-05-6713.


Co-occurrence of organophosphate esters (OPEs) and their degradation products in water and sediment from highly industrialized lake of Korea

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Introduction: Organophosphate triesters (OPEs) have been widely used as flame retardants and plasticizers for diverse industrial and consumer products (Wang et al., 2018). Although degradation products of OPEs have been determined for human biomonitoring in urinary metabolites (Hou et al., 2020), the environmental occurrence and distribution of degradation products were not understood in natural environments. Lake Shihwa, located on the west coast of Korea, is highly contaminated by a variety of organic contaminants. In the present study, OPEs and their degradation products were simultaneously determined for water and sediment samples from Lake Shihwa to assess the occurrence and environmental distribution in the aquatic environments.

Materials and Methods: Forty-one surface water and sediment samples were collected from creek, inshore, and offshore waters of Lake Shihwa in 2021. Fourteen tri-OPEs and degradation products were measured using a high performance liquid chromatography coupled to triple mass spectrometry (LC-MS/MS) with a solid phase extraction (SPE) treatment.

Results: The total concentrations of OPEs in water and sediment ranged from 89.5 to 4760 ng/L and from 3.1 to 3500 ng/g dry weight, respectively. The measured OPE concentrations in creek sediments were recorded as the highest levels around the world. This indicates the significant consumption of OPEs for industrial activities. TEP and TCIPP were predominant in water samples, whereas TEHP was predominant in sediment samples based on hydrophobicity. The total concentrations of degradation products in water and sediment ranged from 16.5 to 1410 ng/L and from 0.25 to 5680 ng/g dry weight, respectively. BCIPHIPP (a degradation product of TCIPP) was predominant degradation products in water and sediments. The highest concentrations of OPEs and degradation products were found in creeks surrounded by industrial complexes and decreased with increasing distances from creeks through inshore to offshore waters. Our findings indicate the major sources of OPEs from industrial activities. The concentrations of OPE in water and sediment were higher than those measured for degradation products, suggesting the influence of ongoing sources. The concentration ratios of degradation products and corresponding OPEs ranged from 0.07 (BCIPP/TCIPP) to 0.93 (BEHP/TEHP) for water and 0.02 (BCIPP/TCIPP) to 0.71 (DEP/TEP) for sediment. The concentration ratios for parent and degradation products could provide a crucial information on the environmental persistence and transformation rate of OPEs in the aquatic environments.

Discussion and Conclusion: OPEs and their degradation products were commonly observed in water and sediment samples, indicating omnipresence of OPEs in the aquatic environments. The significantly highest levels of OPEs were determined in creek sediments directly influenced by industrial activities. OPEs and their degradation products were simultaneously observed as highest levels in aquatic samples, suggesting the major sources of degradation products from the degradation processes of OPEs. Further studies are required for understanding the environmental fate including biotransformation processes of OPEs and their degradation products in the aquatic environment.

Acknowledgments: This study was supported by Korea Environment Industry & Technology Institute(KEITI) through Technology Development Project for Safety Management of Household Chemical Products Program, funded by Korea Ministry of Environment(MOE) (2022002970005)

References:
Levels and Trends (Abiotic)

P-053  Spatial Distribution, Source Identification, and Ecological Risks of Polychlorinated Biphenyls and Organochlorine Pesticides in Surface Sediments from Rivers, Korea

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Introduction
Polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) are persistent organic pollutants that have received global attention (Iakovides et al., 2022). PCBs and OCPs have been detected in environmental samples due to traces of past contamination, disposal activities, illegal use, and industrial byproducts (Iakovides et al., 2022). In Korea, PCBs and OCPs in sediments have been investigated more extensively in marine environments (particularly near large cities or industrial complexes) than in freshwater environments (Choi et al., 2011; Hong et al., 2010). Therefore, this study investigated nationwide contamination of freshwater sediments with PCBs and OCPs in Korea.

Materials and Methods
To determine 65 PCBs and 23 OCPs in freshwater sediments, 77 samples were collected in 2021 from five major river networks. Freeze-dried sample (10 g) was extracted with dichloromethane:hexane (1:1, v/v) using an accelerated solvent extractor (ASE-350; Dionex, USA). The extract was concentrated to 1 mL, cleaned using a multilayer silica gel column, eluted with dichloromethane:hexane (1:9, v/v), re-concentrated to 0.1 mL, and analyzed using a 7890A gas chromatography (Agilent, USA) coupled with a JMS-800D high-resolution mass spectrometer (JEOL, Japan).

Results
Figure shows the concentrations of ∑PCBs and ∑OCPs in freshwater sediments according to sampling site types.

Discussion and Conclusion
The levels of ∑PCBs in industrial sites were the highest, followed by industrial and agricultural, agricultural, and other sites, indicating the higher use of PCBs in industrial sources. The major source of PCBs is the past use of commercial products for transformers and capacitors. Other sources differed depending on dominant PCB homologues: ≤4Cl (i.e., atmospheric deposition and wastewater) and ≥8Cl (i.e., manufacturing processes). The OCP levels in the sediments were not affected by current agricultural sources. The industrial sites showed higher concentrations of ∑OCPs than other areas due to HCB and PeCB that can be generated in industrial processes and incinerators. DDTs and chlordane in sediments are mainly related to their historical use, whereas others are mostly affected by recent inputs. Ecological risks were determined by incorporating the sediment quality guidelines and mean probable effect level quotients. Except for the two samples, the levels of PCBs and OCPs were ecologically acceptable in freshwater sediments.

Acknowledgments
This work was supported by the National Institute of Environment Research funded by the Ministry of Environment (NIER-2021-03-02-037) and Basic Science Research Program through the National Research Foundation funded by the Ministry of Education (NRF-2021R1A6A1A03039572, NRF-2021R1A2C2006517) of the Republic of Korea.

References
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Introduction: Dioxins, representative persistent organic pollutants, continuously remain in the environment and are carcinogenic, mutagenic or toxic to reproduction. Dioxins are emitted into the atmosphere through a variety of anthropogenic activities, including waste incineration and industrial activities. The dioxins released to the atmosphere exist in the gas phase and particle phase, and dioxins in the gas phase move into soil or water through the process of dispersion equilibrium/wet deposition. It is important to characterize atmospheric behavior because most of dioxins are released into the atmosphere and travel through the atmosphere to other environmental media and biosphere. The purpose of this study is to provide basic data for environmental management such as local emission source management. This study was performed to investigate the concentration level, distribution characteristics of gas-particle, and congeners of Polychlorinated dibenzo-p-dioxins (PCDDs) and Polychlorinated dibenzofurans (PCDFs) in atmosphere of Gyeonggi-do.

Materials and Methods: Sampling was performed once a month in 2021 for four regions (Pyeongtaek, Gimpo, Pocheon, Icheon) in Gyeonggi-do using a high-volume air sampler (Active Air Sampler: AAS). Experiments and analysis were performed according to EPA method 1613 and analyzed using high resolution gas chromatograph/high resolution mass spectrometry (HRGC/HRMS).

Results: The average concentration of PCDDs/PCDFs was 0.035 pg I-TEQ/m3 and it was confirmed that all analysis results were below the national air quality standard (0.6 pg-TEQ/m3). The concentration of PCDDs/PCDFs by region ranged from 0.008 to 0.125 pg TEQ/m3 (mean value: 0.038 pg TEQ/m3) in Pyeongtaek, 0.003 to 0.127 pg TEQ/m3 (mean value: 0.035 pg TEQ/m3) in Gimpo, 0.007 to 0.153 pg TEQ/m3 (mean value: 0.048 pg TEQ/m3) in Pocheon, 0.004 to 0.048 pg TEQ/m3 (mean value: 0.018 pg TEQ/m3) in Icheon, respectively. The concentration of PCDDs/PCDFs by region was high in Pocheon (1.087 pg/m3) with various PCDDs/PCDFs emission sources, and the seasonal concentration of PCDDs/PCDFs was low in the summer (0.198 pg/m3) and high in the winter (1.163 pg/m3). The particle-gas distribution of PCDDs/PCDFs was about 84% on the particles and it was confirmed that many PCDDs/PCDFs were distributed in winter particles with low temperatures. Especially lowly-chlorinated congeners that combine 4-5 Cl- were distributed in the gas phase, and highly-chlorinated congeners that combine 6-8 Cl- were distributed in the particle phase. The major contributors on the PCDDs/PCDFs concentration were 1,2,3,4,6,7,8-H7CDF, O8CDF, O8CDD, 1,2,3,4,6,7,8-H7CDD and these congeners contributed about 63% of total concentration.

Discussion and Conclusion: The concentration of PCDDs/PCDFs by region was high in Pocheon (1.087 pg/m3), an urban-rural complex. It’s likely that Pocheon was affected by fugitive emissions such like biomass burning and unregulated open burning. Therefore, it is considered that management of emission sources in this area is necessary. The seasonal concentration of PCDDs/PCDFs was low in the summer (0.198 pg/m3) and high in the winter (0.163 pg/m3). Summer is considered to be affected by wet precipitation due to heavy rainfall, in addition, in the case of winter, it is judged to be affected by stagnant air and increased use of heating fuel. The gas-particle distributions of PCDDs/PCDFs was high in particulate phase. The gas phase increased when the temperature increased and the particle phase increased when the temperature decreased. Previous studies also showed similar results to the gas-particle distributions. As the temperature increases, the PCDDs/PCDFs adsorbed on the particles volatilize into the gas phase and thus the concentration of PCDDs/PCDFs in the particle phase is reduced. The distribution of PCDDs/PCDFs congeners showed that high-chlorinated congeners combined with 6-8 Cl- accounted for 89.2% and it confirmed that most of the high-chlorinated congeners were distributed in the particle phase. As the number of chlorine increases, the Octanol-air partitioning coefficient (KOAC) increases and thus high-chlorine congeners are readily adsorbed onto the particle.

Reference:
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Introduction: Long-term monitoring is essential to investigate air pollution trends at a specific area. In addition, monitoring is an important tool in establishing reduction measures. The objective of this study is to identify the air quality trends in Gyeonggi-do by long-term monitoring data and to provide necessary information for establishing persistent organic pollutants (POPs) management policies by evaluating temporal/regional concentration trends. Atmospheric concentrations of polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) were investigated at residential area, urban-rural complex area, industrial area in Gyeonggi-do from 2013 to 2022.

Materials and Methods: Sampling was performed using a high-volume air sampler (Active Air Sampler: AAS) for eight regions (residential area (Suwon, Gimpo, Icheon), urban-rural complex area (Yongju, Dongducheon, Pocheon), industrial area (Ansan, Pyeongtaek)) in Gyeonggi-do. Experiments and analysis were performed according to EPA method 1613 and analyzed using gas chromatograph/high resolution mass spectrometry (HRGC/HRMS).

Results: The annual average concentration of PCDDs/PCDFs was showed a decreasing trend from 0.450 pg I-TEQ/m3 to 0.024 pg I-TEQ/m3. Since 2014, it was confirmed that the concentration of PCDDs/PCDFs was below the national air quality standards (0.6 pg I-TEQ/m3) in all eight sites. The concentrations of PCDDs/PCDFs by use area ranged from 0.036 pg/m³ to 2.380 pg/m³ (mean value: 0.414 pg/m³) in residential area, 0.037 pg/m³ to 16.313 pg/m³ (mean value: 1.729 pg/m³) in urban-rural complex area, 0.107 pg/m³ to 4.539 pg/m³ (mean value: 0.994 pg/m³) in industrial area, respectively. Despite the less PCDDs/PCDFs emission sources than industrial area, the concentration of PCDDs/PCDFs in urban-rural complex area showed the highest values. The results of seasonal distribution of PCDDs/PCDFs were follow as; Spring - 0.838 pg/m³ (0.062 pg-TEQ/m³), summer - 0.478 pg/m³ (0.039 pg-TEQ/m³), autumn - 1.255 pg/m³ (0.098 pg-TEQ/m³), winter - 1.761 pg/m³ (0.147 pg-TEQ/m³). It was confirmed that the concentration of PCDDs/PCDFs was low in summer and high in winter. The major contributors to the PCDDs/PCDFs concentration were 1,2,3,4,6,7,8-H7CDF, O8CDF, O8CDD, 1,2,3,4,6,7,8-H7CDD and these congeners contributed about 46%–63% of total concentration.

Discussion and Conclusion: The annual average concentrations of PCDDs/PCDFs in atmosphere decreased from 0.450 pg I-TEQ/m3 in 2013 to 0.024 pg I-TEQ/m3 in 2022. As a result of this study, the effect of the policy to reduce persistent organic pollutants emission and the long-term behavior of PCDDs/PCDFs in the environment were confirmed. The concentration of PCDDs/PCDFs by land use was high in urban-rural complex area. It’s likely that urban-rural complex area was affected by various emission sources such as incineration facility and biomass burning. In addition, it is judged that the air diffusion in these regions is difficult due to the basin type. Therefore, it is considered that regulation and management of emission sources in urban-rural complex area can be expected to decrease PCDDs/PCDFs concentration in Gyeonggi-do. The seasonal concentration of PCDDs/PCDFs was low in the summer and high in the winter, and it was confirmed that the distribution of PCDDs/PCDFs congeners showed that high-chlorinated congeners combined with 6-8 Cl accounted for 83.7%. Previous studies also showed similar results to the distribution of PCDDs/PCDFs congeners. In the case of this seasonal difference in concentration, in winter it is considered that the concentration of PCDDs/PCDFs is high due to the effect of increased use of heating fuel and the increase in atmospheric stability due to the occurrence of atmosphere inversion appearance.

Reference:
Levels and Trends (Abiotic)

P-056  Distribution of polychlorinated biphenyls (PCBs), halogenated flame retardants (HFRs), organochlorine pesticides (OCPs), and per- and polyfluoroalkyl substances (PFASs) in sediment from highly industrialized port in Korea

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Introduction: Hexabromocyclododecanes (HBCDDs), polybrominated diphenyl ethers (PBDEs), PCBs, PFASs and OCPs have been used in diverse industries to improve desired properties of products and confer insecticidal effects. Earlier studies have reported that these compounds exhibit high lipophilicity and bioaccumulation in aquatic ecosystem. Due to their persistence, bioaccumulation, long-range transport, and toxicity, certain compounds were designated as persistent organic pollutants (POPs) by Stockholm Convention. Halogenated organic compounds and PFASs used in industry can be released into the surrounding environment, including air and water, during the production, processing, and disposal of products. Pohang New Port, which is surrounded by iron-related industries such as iron, iron ore, and coal industries, is one of the biggest ports in the eastern part of Korea. Due to the physicochemical properties of halogenated organic compounds and PFASs, they have a high affinity for adsorption by sediment particles when introduced into aquatic environments. This study aimed to investigate the relationship between industrial activities and POPs contamination in aquatic environments by measuring halogenated organic compounds and PFASs in port sediments surrounded by diverse industries.

Materials and Methods: In this study, 27 sediment samples were collected from ports located in eastern part of Korea in 2022. The concentrations of PCBs (n=25), OCPs (n=17), PBDEs (n=22), HBCDDs (n=3), and PFASs (n=31) were determined. PCBs and OCPs were identified and quantified using GC/MS, PBDEs using GC/MS/MS, and HBCDDs and PFASs using LC/MS/MS.

Results: In this study, the target compounds were detected in all sediment samples, indicating the ubiquitous contamination of halogenated organic compounds and PFASs in the aquatic environment of the study area. The average concentrations of PBDEs were found to be 1–2 orders of magnitude higher than those of other chemical groups, followed by PCBs, OCPs, HBCDDs, and PFASs. BDE 209 was predominant in almost all sediment samples, accounting for more than 90% of the total PBDEs. The highest total concentration of the target compounds was found to be in the inner part of the port where several wharfs and industries were located. Especially, the other compounds except for PBDEs, showed significantly decrease from the inner part to outer part of port. These results suggest that industrial activities, such as steel production process and waste disposal, may have influenced the spatial distribution of halogenated organic compounds and PFASs. PBDEs had the highest relative distribution in almost all sites, indicating PBDEs was a major industrial chemical in this region. The spatial distribution of PCBs, OCPs, and PBDEs were similar with the total concentration, while HBCDDs exhibited the highest levels in outer part of the port. Pearson’s correlation analysis showed significant positive correlations between the ∼PCB and ∼OCP (r=0.615, p<0.01), ∼PBDE and ∼HBCDD (r=0.799, p<0.01), PBDE and PFASs (r=0.400, p<0.01), suggesting these compounds may have originated from similar source or exhibited environmental behaviors.

Discussion and Conclusion: Concentrations of PCBs, OCPs, PBDEs, HBCDDs and PFASs were measured in sediments from industrialized port of Korea. BDE 209 was identified as the major compound in sediments near industrialized port, and these results fall within the range of pollution levels observed in previous studies. Spatial distribution of target compounds showed that industrial activities could be associated with distribution of halogenated organic compounds and PFASs in aquatic environment. Correlation analysis between target compounds in this study suggested that they have similar contamination source and environmental behavior. Further studies should be conducted to the comprehensive analysis on POPs in aquatic environment and worldwide regional investigation that reflect the industrial characteristic.

Acknowledgments: This research was supported by Development of source identification and apportionment methods for toxic substances in marine environments program of Korea institute of Marine Science & Technology Promotion (KIMST) funded by the Ministry of Oceans and Fisheries (KIMST-20220534)
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Introduction: Polycyclic aromatic hydrocarbons (PAHs) are typical hazardous pollutants associated with ambient particles. Chlorinated polycyclic aromatic hydrocarbons (CIPAHs) have hydrogen substituted by chlorine atoms in PAHs; both have been detected in the environment. Concerning the sources and formation, CIPAHs could be mainly produced by the incomplete combustion process of organic materials in the presence of chlorine, like PAHs¹. In addition, Sankoda et al. suggested that PAHs in tidal flats are possible to transform CIPAHs by photochemical reactions. Here, we focused on PAH-quinones (PAHQs) because PAHQs in the air have been known to be produced by not only primary formation via the incomplete combustion processes but also secondary formation via photochemical reaction of PAHs with reactive oxygen species. Therefore, PAHQs are expected to be used as organic tracers for the secondary formation. In this study, we investigated the behaviors of CIPAHs and PAHQs in ambient particles through summer days. To evaluate the relationships between CIPAHs and certain PAHQs concentrations, we discussed the possibilities of CIPAH formation via photochlorination of PAHs on ambient particles.

Materials and Methods: The air particle samples were continuously collected on quartz fiber filters (QFFs) in every 2-hour for nine days in an urban site of Nagoya, Japan through July (summer) 2017. The extraction procedures of QFFs were performed by dichloromethane including internal standards using ultrasonication and the extracts were concentrated by N2 purge. Target compounds in the extracts were analyzed by GC-Orbitrap MS. The detailed analytical conditions were described elsewhere³.

Results and Discussion: During the daytime, the temporal variation of ΣPAHQs showed a similar trend to that of water-soluble organic carbon (WSOC) that is a typical organic tracer of secondary produced particles. Among PAHQs, the concentrations of WSOC showed significant correlations (p<0.05) with those of 1,8-naphthalic anhydride (Napth Anhy) and diphenic anhydride (Diphe Anhy). These PAHQs were principally produced from photo irradiation of corresponding parent PAHs in organic solvent, which remained for further photo irradiation (data not shown). Therefore, Napth Anhy and Diphe Anhy could be considered as useful tracers in PAHQs to evaluate the secondary formation. The relationships of concentrations among certain CIPAHs and tracer PAHQs during the daytime and nighttime hours is shown in Table 1. The concentrations of 9-chlorophenanthrene (9-CI-Phe) and 6-chlorobenz[a]pyrene (6-CIBaP) showed significant correlations (p<0.05) with tracer PAHQs during daytime hours (Table 1. A), but not during nighttime hours (Table 1. B). It suggests that the concentrations of these CIPAHs could be related to photo irradiation, so that a portion of CIPAH are possible to be produced by photochlorination of PAHs in ambient particles.


Table 1 Correlation coefficient (upper portion) and p-values (downer portion) among CIPAHs and tracer PAHQs concentrations during the (A) daytime (n=54) and (B) nighttime (n=54).

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<th>9-CIPhe</th>
<th>9-ClAnt</th>
<th>1-CIPy</th>
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<td>6-CIBaP</td>
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<td>Diphe Anhy</td>
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Introduction: Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous toxic organic compounds originated from anthropogenic activities. The major pollution routes and/or sources of PAHs are atmospheric deposition, petroleum release, and incomplete combustion from numerous industrial and domestic activities in the coastal environments. Long-term trends and spatial variation of PAHs are essential to understand the environmental process, source emissions, regulation effectiveness, and the subsequent health risks. Masan and Haengam Bays, located on the south coast of Korea, are characterized by semi-enclosed coastal regions. Earlier studies have reported long-term trends of 16 PAHs, proposed by the US Environmental Protection Agency (US EPA), in sediment from these regions. However, limited studies have been conducted for long-term trends of PAHs in multiple environmental matrices simultaneously collected from these bays. In our study, the comprehensive monitoring of PAHs in seawater and sediment samples from Masan and Haengam Bays to investigate long-term trends and spatial variation of PAHs in the coastal environments.

Materials and Methods: Three surveys in Masan and Haengam Bays were conducted 8 years apart; 40 seawater and 54 sediment samples were collected from approximately the same location in 2005, 2013, and 2021. Sixteen PAHs consisted non-alkylated PAHs were analyzed using gas chromatography coupled to a mass spectrometer under the selected ion monitoring mode.

Results: Except for InP, all PAHs were detected in seawater and sediment during the three surveys. Seawater and sediment samples showed different long-term trends. The total concentrations of 16 PAHs in seawater increased over time, whereas those in sediments declined rapidly after 2005. Spatially, the concentration of PAHs in seawater was highest at Masan Bay in 2005 but later high at Haengam Bay and offshore, while the concentration in sediment was highest at Haengam Bay from 2005 to 2013 but highest at Masan Bay in 2021. The measured concentrations of PAHs did not exceed the sediment quality guidelines, such as ERL, ERM, and PEL, proposed by international authorities. In this study, matrix-dependent distribution was observed for PAHs due to their physico-chemical properties. The relative contribution of lower-molecular-weight PAHs was higher in seawater samples, whereas the higher-molecular-weight PAHs were dominant in sediment samples. Based on diagnostic ratios and the PCA-MLR model suggested the co-existence of pyrolytic and petroleum contamination sources for PAHs. The contribution of petrogenic sources to PAH contamination increased from 2005 to 2021 in seawater samples, while PAHs in sediment mainly originated from combustion sources.

Discussion and Conclusion: The long-time trends of PAH concentration, profile, and sources were investigated in multiple environmental matrices from semi-enclosed bays. Distinct temporal trend differences in PAHs between seawater and sediments indicate industry-scale changes and the effect of polluted sediment remediation policies implemented since 2005. Regional differences in PAH pollution state may be related to the type and size of adjacent industries and environmental factors such as dissolved oxygen and circulation in seawater. Our results suggest the major source of PAHs in seawater has changed from pyrolytic sources to petrogenic-related sources. Although the measured PAHs did not exceed the sediment quality guidelines, PAHs should be continuously monitored for marine ecological risks.

Acknowledgments: This research was supported by Development of source identification and apportionment methods for toxic substances in marine environments program of Korea institute of Marine Scince & Technology Promotion(KIMST) funded by the Ministry of Oceans and Fisheries(KIMST-20220534).
**Levels and Trends (Abiotic)**

**P-059  Air Concentrations of Polychlorinated Biphenyl (PCB) During Large Transformer Dismantling**

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**Introduction:** Some transformers containing PCBs in Japan are too large and heavy to be transported to treatment facilities, necessitating on-site dismantling. This study aimed to measure airborne PCB levels during such operations at PCB waste storage sites to ensure safety and enhance efficiency.

**Materials and Methods:** We designated two air sampling points: inside the PCB storage area (the 17th floor of a commercial building in Tokyo), where dismantling occurred, and outside the storage area within the building. Sampling was conducted between July 2020 and February 2022, capturing a 24-hour period encompassing pre-, during-, and post-dismantling phases. Inside the storage area, air samples were collected using a low-volume air sampler with a cartridge at a flow rate of 2 L/min. During dismantling, the transformer was enclosed by a hazard containment tent with a front room between it and the storage area to maintain negative pressure. The air from the tent was filtered through activated carbon and then exhausted. Outside the storage area, air sampling was performed using a high-volume sampler with quartz fiber filter paper, polyurethane foam, and activated carbon fiber felt at a flow rate of 700 L/min. Samples were cleaned up after extraction and analyzed by GC-HRMS. The target substance was each PCB homologue from Mono- to Deca-CB. PCB concentration, corrected for temperature effects, was calculated using the equation below. We estimated the temperature-dependence coefficient (ki) using panel data analysis described in Koshiba et al. (2018) based on our previous measurements of PCB homologue concentrations at PCB waste storage facilities.

\[
C_{20,i} = C_{T,i} \cdot \exp \left\{ -k_i \left( \frac{1}{293.15} - \frac{1}{273.15 + T} \right) \right\}
\]

where \(C_{(20,i)}\): corrected PCB concentration at 20 °C, \(C_{(T,i)}\): measured PCB concentration at \(T\)°C, \(k_i\): coefficient for temperature dependence, \(T\): air temperature in PCB waste storage site, \(i\): PCB homologue.

**Results:** The highest total PCB concentration (sum of all homologues from Mono- to Deca-CB; 51,000 ng/m³) inside the tent was observed during the transformer demolition. Other dismantling stages revealed concentrations between 36-400 ng/m³, exceeding pre-dismantling levels. Upon dismantling completion, the levels dropped to 9.2-72 ng/m³, below the pre-dismantling levels.

![Fig. 1 Measured and temperature-corrected air PCB concentrations inside the PCB waste storage before and after on-site dismantling.](image)

**Discussion and Conclusion:** The total PCB concentrations remained below the Japanese workplace control standards (0.01 mg/m³) and provisional air quality levels (500 ng/m³), except during demolition. Although PCB levels spiked during demolition, the high concentrations were limited to within the tent, with workers wearing protective masks. The exterior of the PCB storage site displayed a total PCB concentration of 110 ng/m³ during demolition, which complied with safety standards. The study observed a trend of reduced PCB concentrations after dismantling. Temperature correction revealed the decreasing trends of PCB concentrations after completion.

**Acknowledgments:** This research was supported by the Japan Environmental Storage & Safety Corporation. We thank the PCB storage facility for their support in sampling.

Levels and Trends (Biota)

P-060 Time trends of extractable organohalogen (EOX) in archived animal samples

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Introduction: Persistent organic pollutants (POPs) and halogenated compounds which have similar risks are increasing, so extractable organohalogen (EOX) is attracting attention as a comprehensive assessment. POPs are known to bioaccumulate in higher trophic animals. This study is conducted to understand the temporal changes of EOX in archived finless porpoise blubber samples and kite liver samples separately by chlorine (EOCl) and bromine (EOBr) elements and by molecular weight. By comparing the concentration of POPs reported in previous studies, unidentified halogens were quantitatively measured.

Materials and Methods: Blubbers of finless porpoise from individuals that drifted to the Seto Island Sea in 2001-2016 and livers of kite from individuals collected at Matsuyama Airport in 1977-2018 were used. Each sample was kept frozen at -25°C at the Environmental specimen bank for global monitoring (es-BANK) in Ehime University. The extraction method followed previous studies. Samples were extracted in organic solvents and washed. Extracts were fractionated at a molecular weight of 1000 g/mol (lower fraction is EOCl-L or EOBr-L). Samples were irradiated for 15 min with a thermal neutron at KURNS (Kyoto University). Concentrations in the samples were calculated using the comparison method between those and standard samples.

Results and Discussion: EOCl-L was 17.2 ± 13.4 µg/g lipid, and EOBr-L was 1.91 ± 0.99 µg/g lipid and 0.4 times decreased from 2001 to 2016 in finless porpoise samples (Figure 1). In kite samples, EOCl-L was 15.9 ± 18.9 µg/g lipid and EOBr-L was 1.59 ± 0.96 µg/g lipid, and decreased when comparing 1977 and 2018.

In finless porpoise, the percentage of PCBs and OCPs in EOCl-L was about 80% and exceed 100% in some individuals. This suggests that naturally occurring chlorine in the blubber is low, and unidentified chlorine is most likely of anthropogenic origin. In kite, EOCl-L decreased from 1977 to 1990, possibly reflecting a decreased in PCBs due to environmental measures. Unidentified chlorine was about 50%, and there was difference in accumulation from blubber of finless porpoise. Unlike chlorine, 70% of EOBr-L was unidentified bromine in both of finless porpoise and kite.

The results of this study enabled temporal and quantitative evaluation of EOCl-L and EOBr-L, and unidentified chlorine and bromine in finless porpoise blubber and kite liver samples. Further investigation of unidentified compounds and regular monitoring of EOX-L will be important in the future.

Acknowledgments: This research was supported by a Grant-in-Aid for Scientific Research (A) from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), (Project Number: 20H00646).

References:
Levels and Trends (Biota)

P-061  Species-specific accumulation and body burden of persistent organic pollutants (POPs) in marine wild species from Korean coastal water

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Introduction: Marine ecosystems have been threatened by various types of toxic pollutants. Exposure of persistent organic pollutants (POPs), including polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), and polychlorinated naphthalenes (PCNs), and chlorobenzenes (CLBz), to wildlife species are a major global concern, as indicated by declining populations. Despite this, data is scarce on bioaccumulation processes and partitioning of POPs in tissues and organs from various wildlife species of Korea. In this study, POPs were analyzed in the tissues and organs of marine mammals (cetaceans and pinnipeds) and turtles collected from Korean coastal waters to assess the occurrence, species-dependent accumulation, tissue-specific distribution, and body burden of POPs.

Material and Methods: Marine mammals (n=10) and turtles (n=2) were obtained from bycaught samples along the Korean coasts. The samples were transported to the Cetacean Research Institute, Ulsan, Korea for dissection. Before dissection, biological measurements such as body size, weight, and gender were determined. Major organs or tissues obtained in our study were blubber, fat, muscle, heart, liver, kidney, lungs, stomach, intestine, melon, and brain. All samples were extracted using a Soxhlet extractor and then passed through gel permeation chromatography to remove lipid contents in the samples. Multi-layer silica gel column was used for the cleanup of interferences in the extracts based on the previous studies. PCBs, OCPs, and CLBz were measured for gas chromatography coupled to a mass spectrometer (GC-MS) and PCN for tandem mass spectrometer (GC-MS/MS).

Results: In this study, many congeners of OCPs, PCBs, CLBz, and at least one congener of PCNs were discovered in the tissues and organs of all marine mammal species and turtles, with significant contributions from p,p’-DDE (Range: 1-12640 ng/g lw), CB 153 (Range: 0-6539 ng/g lw), CB 138 (Range: 0-3931 ng/g lw) Among the target organs and tissues of marine mammals, the concentrations of POPs in blubber and melon showed several times higher than those in other organs. POPs have been detected in higher concentrations in organs and tissues other than fat in turtles. Among all target analytes, OCPs, such as DDTs, showed the highest concentration in major tissues and organs. Inter- and intra-species differences in the body distribution of POPs were observed in our study. All the analyzed POPs concentrations in tissues or organs from pinnipeds and turtles were lower than those found in cetaceans. The median concentration of pp’-DDE was 18 ng/g lw in turtles, 1400 ng/g lw in pinnipeds, and 1440 ng/g lw in cetaceans. Among the two predominant PCB congeners, the median concentration of CB 153 in turtles was 1 ng/g lw, pinnipeds show 582 ng/g lw, and in cetaceans 693 ng/g lw. Whereas the median concentration of CB 138 in turtles was zero, in pinnipeds 318 ng/g lw, and in cetaceans 426 ng/g lw. In our study, the POPs concentrations found in our study did not exceed the reference values associated with ecotoxicological concerns. The estimated body burden of POPs in marine mammals was mostly (>90%) governed by blubber.

Discussion and Conclusion: Marine mammals and turtles from Korea contained several levels of PCBs, OCPs, CLBz, and at least one congener of PCNs. The contamination of POPs in organs with high lipid content showed the lipophilic characteristics of POPs. The influence of lipid mobilization in turtles results in the redistribution of POPs in organs and tissues other than fat. The lower concentration of analyzed POPs in pinnipeds and turtles than in mammals is probably due to different diets, habitats, and metabolism. The predominant presence of p,p’-DDE indicates a greater resistance and biomagnification in the food web. Age and sex were confounding factors governing POPs accumulation and body distribution within species. Intra- and inter-species differences in the POPs concentrations were observed due to probably age, sex, diet, and metabolisms. Although the POPs concentrations were below the threshold values, the continuous monitoring of POPs is required for chemical exposure and conservation issues. The body burden of POPs was first calculated from various species of marine wildlife.

Acknowledgment: This work was supported by the project entitled ‘Development of Techniques for Assessment and Management of Hazardous Chemicals in the Marine Environment’, funded by the Ministry of Oceans and Fisheries (MOF), Korea.

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Persistent Organic Pollutants (POPs) are widely distributed throughout the environment. Once these toxic contaminants reach the surface water, they may concentrate in suspended particulate, sediments and bioaccumulate in fish. Polychlorinated dibenzo-p-dioxins (PCDDs), Polychlorinated dibenzofurans (PCDFs), Polychlorinated biphenyls (PCBs), Polybrominated diphenyl ethers (PBDEs), and Polybrominated biphenyls (PBB) are lipophilic organic contaminants with high potential for bioaccumulation. Rio Grande reservoir is part of the largest and most important water body in the São Paulo Metropolitan Region, the Billings complex. For many years it presented low environmental quality due to contamination with organic sewage from the Metropolitan Region. The Rio Grande reservoir is used for drinking water supply and for fishing, especially by local population. Fish monitoring serves as an important indicator of water quality problems and of contaminated sediments, as well as information regarding fish consumption, and may suggest the necessity of chemical analyses to evaluate specific contaminants as part of the comprehensive water quality monitoring program of CETESB (São Paulo State Environmental Protection Agency). Therefore, three fish species (Astyanax sp, Hoplias malabaricus, Geophagus brasiliensis) with different feeding habits and trophic web positions were caught and analyzed for their levels of PCDDs, PCDFs, PCBs, PBDEs and PBB-153 in muscle tissue and visceral material.

In 2020, 65 fish were collected in two sites, one located in the riverine zone (entrance) and the other in the lacustrine zone (end of reservoir) in the Rio Grande reservoir. Fish muscles and visceral material were separated before analysis. Fish samples of the same species were composite to obtain more mass and a better spatial coverage at each sampling site. Muscle and visceral material were lyophilized until constant weight and further ground to obtain a fine powder. The samples were spiked with 13C12-PCDD/F, 13C12-PCB and 13C12-PBDE surrogate standards and extracted in a microwave extractor with toluene:acetone (8:2). Further clean-up steps were performed using different columns: a multilayer silica column (40% H2SO4 and 10% AgNO3) was connected in series with an alumina column, followed by a third carbon column. PCDD/F, PCB, and PBDE final extracts were analyzed by gas chromatography coupled in a high resolution magnetic sector mass spectrometer. GC was fitted with a VF-Xms capillary column (60m x 0.25mm id x 0.25µm) for PCDD/Fs, an HT8-PCB capillary column (60m x 0.25mm id x 0.25µm) for PCBs and a RTX-1614 capillary column (15m x 0.25mm id x 0.10µm) for PBDEs and PBB-153. PCDD/F, PCB and PBDE quantification were performed according to the methods US EPA 8290A, US EPA 1668C and US EPA 1614A, respectively.

The results showed detectable levels of the target compounds in almost all samples. Concentrations were 7 to 55 higher in visceral material than in muscle tissue, indicating a recent contamination. The collected organs were liver (hepatopancreas in some fishes), kidney and spleen, reflecting not only the latest body metabolism, but also the beginning of the processes of bioaccumulation and biomagnification of contaminants. Overall, the highest concentrations found were of PCBs, followed by PBDEs, PBB-153 and PCDD/Fs. The indicator PCBs corresponded to 66-78% of PCBs in the tissue and visceral samples. Between the 20 PBDEs congeners analyzed, the predominant were PBDE-47, -99, -100, -153, -154, -209, the same congeners used in commercial mixtures. The predominance of PCDF over PCDD was 82-100% and the congener 2,3,7,8-TCDD was found only in the visceral material.

The POPs concentrations quantified in the fishes from the entrance site of the reservoir presented the highest contamination. Fish from this location reflect the water and sediment quality resulting of the discharge of liquid effluents from both domestic and industrial sources. Regarding the trophic level distribution of contaminants, as expected concentrations showed a trend to increase from the lower to higher positions (Astyanax sp > Geophagus brasiliensis > Hoplias malabaricus), suggesting the biomagnification process. In the present study an approximately ten-fold reduction of indicator PCBs concentrations in Astyanax sp (from Σ7PCBmuscle= 57,8 µg/kg in 2009 to 4,80 µg/kg in 2020) and Hoplias malabaricus (from Σ7PCBmuscle= 76,4 µg/kg in 2009 to 5,73 µg/kg in 2020) was obtained, when compared to a former study from 2009, in the same location3, indicating a possible declining trend for this class of compounds.

We gratefully acknowledge the support and infrastructure provided by CETESB and all the people who worked on this study. The authors also acknowledge the funding from State Water Resources Fund (FEHIDRO), grant number 104/2015 - AT-656.

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1. Introduction:
Since the early 20th century PCN have been produced as industrial chemicals and have been unintentionally spread into the environment. Because of their persistent and bioaccumulative properties, and a comparable toxicity to dioxins, they were banned from production and usage since the early 1980s1,2,3.

2. Materials and Methods:
Sampling: From August to October 2022, a total of 344 individual fish samples were collected in 35 streams and rivers in North-Rhine-Westphalia (Germany) and pooled into 35 samples. Each sample was covered by one fish species (roach, Rutilus rutilus; brook trout, Salmo trutta fario; perch, Perca fluviatilis; chub, Squalius cephalus; common dace, Leuciscus leuciscus) of which 5 to 12 fish were merged to one pool, respectively.

Analysis: The fat was extracted from the homogenized fish fillets and six 13C10-labelled PCN were added. After a 3-step cleanup (sulfuric acid/silica gel-, alumina oxide- and activated charcoal-column) using a DEXTech Pure apparatus (LCTech GmbH, Germany) the PCN were determined by using HRGC-HRMS (Agilent 6890 equipped with a DB-5 column; 60 m; 0.25 mm i. D.; 0.10 µm f. d. coupled to a Waters Autospec Ultima HRMS at a resolution of 10,000 in SIR-mode. The order of the PCN listed is in all cases the order of their elution.

3 Results:
The congeners PCN42, PCN-28/36, PCN27, PCN46, PCN-52/60, PCN50, PCN53, PCN49, PCN-66/67, PCN-64/6, PCN69, PCN-71/72, PCN65, PCN70, PCN73, PCN74 and PCN75 were determined of which the stated pairs could not be chromatographically separated.

Measured at the relative levels to each sample the congeners, PCN42, PCN-71/72, PCN53, PCN69, PCN46, PCN-66/67 and PCN-64/68 showed the highest levels in this order. These congeners which represent 93.9 % by amount of the determined congeners were examined more closely in the following considerations.

Table 1: Ranges of findings in all samples. Amounts in ng/kg w/w

<table>
<thead>
<tr>
<th></th>
<th>PCN-52/60</th>
<th>PCN42</th>
<th>PCN-71/72</th>
<th>PCN53</th>
<th>PCN69</th>
<th>PCN46</th>
<th>PCN-66/67</th>
<th>PCN-64/68</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min</td>
<td>1.9</td>
<td>1.6</td>
<td>&lt; LOD</td>
<td>0.3</td>
<td>&lt; LOD</td>
<td>&lt; LOD</td>
<td>0.3</td>
<td>&lt; LOD</td>
</tr>
<tr>
<td>Max</td>
<td>189</td>
<td>129</td>
<td>35.0</td>
<td>12.5</td>
<td>38.4</td>
<td>11.4</td>
<td>23.6</td>
<td>15.6</td>
</tr>
<tr>
<td>LOD</td>
<td>0.20</td>
<td>0.16</td>
<td>0.09</td>
<td>0.25</td>
<td>0.09</td>
<td>0.25</td>
<td>0.02</td>
<td>0.11</td>
</tr>
<tr>
<td>Mean</td>
<td>20.4</td>
<td>17.3</td>
<td>5.2</td>
<td>3.5</td>
<td>4.4</td>
<td>2.3</td>
<td>2.9</td>
<td>2.1</td>
</tr>
<tr>
<td>Median</td>
<td>11.1</td>
<td>10.0</td>
<td>2.0</td>
<td>2.1</td>
<td>1.6</td>
<td>0.9</td>
<td>1.3</td>
<td>0.8</td>
</tr>
<tr>
<td>90th Perc.</td>
<td>40.5</td>
<td>27.7</td>
<td>15.0</td>
<td>8.9</td>
<td>11.1</td>
<td>5.9</td>
<td>4.8</td>
<td>5.4</td>
</tr>
<tr>
<td>95th Perc.</td>
<td>53.5</td>
<td>43.7</td>
<td>18.0</td>
<td>10.3</td>
<td>14.7</td>
<td>7.1</td>
<td>10.4</td>
<td>6.2</td>
</tr>
</tbody>
</table>

In all of the samples analyzed PCN-52/60 and PCN 42 were the most abundant congeners. Their ranges of levels in perches and roaches are separated and completely above those of the other PCN. This is remarkable since the samples of these two species were derived from throughout the whole state and consisted of 8 samples containing 77 fish (perches) and 5 samples containing 52 fish (roaches) respectively.

Furthermore, there were 85 brook trout in 9 samples, 120 chubs in 12 samples and 10 common daces in one sample. This single sample showed levels between 2.5 and 20.1 ng/kg w/w and a comparable pattern to the other samples.
Levels and Trends (Biota)

P-064  Polychlorinated Naphthalenes (PCN) in Fish from Running Waters In North-Rhine-Westphalia (Germany)

Figure 1: Box plots of PCN concentrations in trout and perch. Amounts in log (ng/kg w/w).

Figure 2: Box plots of PCN concentrations in chubs and roach. Amounts in log (ng/kg w/w).
4. Discussion:
In one study, PCN were determined in a number of fish species caught in the Bothnian Bay and Bothnian Sea. There, the congeners 66/67 were the most abundant PCN in perch followed by 52/60, 33/34, 61 and 69.

In another study, PCN were measured in lake trout collected from Lakes Huron and Michigan and Siskiwit Lake. The congeners 52/60, 66/67, 69, 61, 64/68 and 71/72 showed the highest abundance (in descending order).

5. Conclusions:
The cleanup procedure described here provides PCDD/F, non-ortho PCB, PBDD/F and PXDD/F in the same fraction of PCN as well as mono- and di-ortho-PCB and PBDE in a 2-step cleanup omitting the activated charcoal step.

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Introduction: Migratory shorebirds migrating along the East Asian-Australasian Flyway (EAAF) are already threatened by climate change and high rates of land reclamation (Melville, Chen, & Ma, 2016). As a result, species in the flyway are experiencing population declines of up to 8% per year (Clemens et al., 2016). Potentially compounding these problems is the threat of environmental contamination from persistent organic pollutants (POPs). The lipophilic properties of many POPs cause them to be stored in adipose tissue which these birds use as fuel stores. Paired with physiological stress from migration, these considerations justify investigations into POPs contamination in shorebirds of the EAAF, and any possible interspecific differences therein. However, few studies have previously targeted POPs in shorebirds of this endangered flyway.

Materials and Methods: In this study, we analysed liver samples from 42 individuals across five different Arctic-breeding species of shorebirds, including bar-tailed godwits (Limosa lapponica), broad-billed sandpipers (Limicola falcinellus), great knots (Calidris tenuirostris), curlew sandpipers (Calidris ferruginea) and red-necked stints (Calidris ruficollis). All studied birds died from physical trauma while on their non-breeding grounds in Australia and were opportunistically sampled between 2009 and 2020. We targeted 48 compounds including 21 PCB congeners, 5 chlordanes and their metabolites, DDT and its metabolites, hexachlorobenzene, 9 PBDEs brominated flame retardants, 2 methoxylated PBDEs (2-MeO-BDE68 and 6-MeO-BDE87), syn- and anti-Dechlorane Plus (s/a-DP), 2,4,6-tribromophenol (TBP), tetrabromobisphenol A (TBBPA), and 3 hexabromocyclododecane isomers (−,-,− and −HBCDs).

Results: The most detected pollutants were TBP (detection frequency 100%), followed by p,p′-DDE (95%), PCBs (17-95% depending on the congener), hexachlorobenzene (81%), oxychlordane (74%), alpha-hexabromocyclododecane (64%) and tetrabromobisphenol A (50%). In descending order, median concentrations of all pollutants with DF > 50% followed the order DDTs (overall median 327 ng/g lw) > PCBs (35.4 ng/g lw) > TBP (23.7 ng/g lw) > oxychlordane (5.7 ng/g lw) > HCB (3.6 ng/g lw) > −HBCD (0.55 ng/g lw) > TBBPA (0.35 ng/g lw). Only limited significant differences were found between species, except for TBP where curlew sandpipers showed higher concentrations than every other species.

Discussion and Conclusion: Compared to existing studies in POPs in shorebirds from other flyways, our observed POPs concentrations in shorebirds were generally equivalent to those established in the literature (Braune & Noble, 2009), if not higher than those previously noted (Schwemmer et al., 2015). Nevertheless, very few individuals exhibited pollutant burdens approaching health concern thresholds, except for 11 individuals from three species with DDT liver concentrations higher than thresholds established for eggshell thinning. Perhaps most notable in our results was the elevated TBP concentrations in curlew sandpipers. As the fastest declining of all species in the EAAF, this pollutant pattern in these sandpipers is particularly notable. Little is yet known about the sources and toxicology of this compound in birds, and evidently more research is needed in this area.

References:
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Introduction: PolyChlorinated Naphthalenes (PCNs) is a group of 75 mono- to octachlorinated compounds, historically produced for a wide range of applications due to their versatility and unintentionally released through a diversity of thermal processes, that are continuously being uncovered. In line with their classification as POPs in 2015, the community is expressing renewed interest in PCNs. Despite this, risk assessment is hampered by the insufficient amount of occurrence and exposure data. Furthermore, the level of knowledge acquired is substantially lower compared to the more famous polychlorinated dibenzodioxins/furans (PCDD/Fs) and polychlorinated biphenyls (PCBs), although higher chlorinated PCNs also present the capacity to exert dioxin-like (dl) effects. In this context, the present work aimed at providing simultaneous occurrence data for PCNs, PCDD/Fs and PCBs in food products recently acquired on the French market. Frequently consumed items were selected to provide a representative evaluation of the consumer’s exposure to PCNs, in regards with the other dioxin-like POPs studied.

Materials and Methods: Sixty food items representative of the consumption of the French population were acquired in 2021 in supermarkets or a local market, and included fish, meat, milk and dairy products, eggs, baby foods and vegetable oils. Sample preparation was performed simultaneously for PCNs, PCBs and PCDD/Fs, enabled by the structural similarity existing between these substances. Briefly, it involved a pressurized liquid extraction followed by an automated purification and an instrumental analysis by gas chromatography coupled to high-resolution mass spectrometry. 69 PCNs, 18 PCBs, and 17 PCDD/Fs were quantified using isotopic dilution, and exposure was assessed by linking the concentrations and the toxic equivalents (TEQs) to the consumption data of adults obtained from the third French individual and national food consumption survey.

Results: PCNs were detectable in all the food items analyzed, with levels ranging from 2.5 pg g⁻¹ wet weight (ww) for ready-to-eat meals intended for toddlers to 150 pg g⁻¹ ww for canned fish products. The measurement of almost all the congeners allowed to demonstrate the predominance of lower chlorinated homologs (24 Cl, contributing to ~70 % of total PCNs concentrations). In addition, the simultaneous measurement of PCNs with PCDD/Fs and PCBs showed a low but non-negligible contribution of PCNs to total POPs concentrations (0.9 – 50 %, 9% on average), decreasing when considering the TEQs (0.4 – 24 %, 5 % on average). The dietary intake calculated for PCNs arose mostly from the consumption of meat and meat products followed by milk and dairy products (74 – 90 % of the total PCNs intake), and was far below the TEQ-related tolerable weekly intake provided by the EFSA (2 pg TEQ/kg bw/week).

Discussion and Conclusion: Ubiquity of PCNs already demonstrated at a global scale was confirmed in France, a country where these chemicals were historically produced. Although the levels of PCNs found in this study are in the range of those measured in other countries in Europe, the predominance of di- to tetraCNs, not frequently targeted in other works, suggests that more attention should be paid to these lower chlorinated congeners, especially as they can reflect unintentional sources of PCNs, known to have increased in recent years. Thanks to the concomitant measurement of PCNs, PCBs and PCDD/Fs, the relative contributions to the dietary intakes could be evaluated; that of PCNs was found to be much lower and the associated risk to human health appears to be low. However, the high contributions of PCNs concentrations to total PCNs+PCBs+PCDD/Fs concentrations (up to 50% for vegetable oils), such values being never reported before, demonstrates that PCNs can substantially add to the cocktail of dioxin-like contaminants.

Acknowledgments: This work was supported by the Human nutrition and food safety department of the French National Research Institute for Agriculture, Food and Environment (AlimH, INRAE) [Grant HALOEXPO].

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2. Godéré et al., Chemosphere, 2022, https://doi.org/10.1016/j.chemosphere.2022.136563
Introduction: Brominated flame retardants (BFRs) are organobromine compounds used to prevent or slow down the ignition of consumer goods. Polybrominated diphenyl ethers (PBDEs) were used for this purpose for several decades, and after the ban on their use, the demand for novel brominated flame retardants (nBFR) increased. PBDE and nBFR tend to leak from customers' goods. They are persistent in the environment and have the potential for biomagnification in the food chain. The toxicity potential and omnipresence of BFRs in food of animal origins have been demonstrated. The European Commission released the recommendation for 5 classes of BFR monitoring in food of animal origin (2014/118/EU). However, food of animal origin safety is strongly related to feed quality, so the aim of the study was BFRs content (10 PBDE congeners and 8 nBFRs) determination in feed and feed materials.

Materials and Methods: All used solvents were of a high purity suitable for residue analysis. Analytical standard solutions of their labelled homologues of ten PBDE congeners (BDE-28, 47, 49, 99, 100, 138, 153, 154, 183 and 209) and eight nBFR: 2,3,5,6-tetrabromophenol (TBX), 2,3,4,5,6-pentabromotoluene (PBT), heksabromobenzene (HBB), pentabromoethylenbenzene (PBB), 2-ethylhexyl-tetrabromobenzene (EH-TBB), 1,2bis(2,4,6-tribromophenoksy)lethanol (BTBPE), bis(2-ethylhexyl) tetrabromophobtalanle (BEH-TBPH), 1,2bis(pentabromophenoxo)lethane (DBDPE) were used. High-resolution gas chromatography coupled with high-resolution mass spectrometry (HRGC-HRMS) was used for analyte quantification. The fifty-nine feed and feed materials were taken by Veterinary Inspection Collection as part of the national official control (National Official Feed Control Schedule) in 2022. The samples are animal fats (19), vegetable oils (4), fish meals (10), feed for fish and pets (6), and compounds feed (20).

Results: Almost all (98.3%) samples were polluted with at least one of the tested PBDE congeners, and nearly 90% of samples were contaminated with nBFRs. The fishmeal was the matrix with the higher PBDEs detection frequency. The most frequently quantified PBDE congener was BDE-209 (93%), followed by BDE-47 (90%). BDE-138 was not found in any sample. BDE-183 was quantified only in 8% of samples. ∑4 PBDE content was in the range of 0.42 – 3.2 ng/g of feed with 12% moisture with an average of 0.66 ng/g. The highest level was in sunflower oil and the lowest in compound feeds. The most frequently detected nBFR was PBFR (68%), followed by DBDPE (64%). Also commonly found was HBB (46%). PBB and EH-TBB were quantified only in single samples. The average content of ∑8BFRs was 0.29 ng/g within the range of 0.04 – 3.1 ng/g. Compound feed and animal fats were the lowest and the highest polluted by nBFRs feed categories, respectively.

Different patterns were observed between feed categories. BDE-209 and DBDPE were the most abundant congeners except fishmeal and feed with pets with dominant content of BDE-47. The median DBDPE content was higher than BDE-209 in animal fats and compound feeds for hens. Additionally, a substantial share of BEH-TBPH was found in the compound feeds for pigs, fishmeal, and feeds for fish and pets.

Discussion and Conclusion: All samples were polluted by at least one BFR. Similar and 5-times higher PBDE levels in fish meals were reported from France and another world regions. Concentrations of a large set of brominated flame retardants (BFRs) and other world regions which are lipophilic compounds that have been widely applied after the phasing-out of legacy BFRs, can bioaccumulate through the food chain. However, information on NBFs in animal feeds, the beginning of farm-to-fork pathway, is very limited. Fishmeal is one of the most widely applied feedstuff worldwide. The present study identified eleven nBFRs from ninety-two globally collected fishmeal samples with levels in the range of 0.13 – 822 (mean: 15.1 ± 85.5) indicated nBFR levels in fishmeal several times or even hundreds of times higher than our results. PBDEs use has been banned, but these substances are still present in the feed. In addition, traces of nBFR can already be found. Considering the food quality of animal origin, it is reasonable to undertake feed monitoring for traces of BFRs.

Acknowledgments: Thanks to the staff of the Department of Radiobiology for their commitment to performing the study.

References:
P-069 Characterization of congener patterns of PCDD/F and PCBs in feed

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Introduction: Dioxin contamination incidents in food demand immediate attention and concerted efforts to perform harm reduction. By studying the congener patterns and trends associated with dioxin contamination, effective measures can be developed, early detection strategies and risk management protocols can be applied to safeguard public health and maintain the integrity of food supply. The causes contributing to these incidents include the use of contaminated feed, environmental or soil pollution, and improper food processing practices. Contaminated feed is related to be the major source of contamination for animal products. Implementing a surveillance program on feed and feed ingredients is a great tool to avoid contamination on the food chain. A long term surveillance can provide useful information regarding the most critical animal feed supplies. Additionally, information related to the PCDD/F and PCBs congeners profile can be accessed and can be strategically used in cases of food contamination incidents to identify the contamination source.

Materials and methods: Data from the feed surveillance program performed by the Brazilian Ministry of Agriculture, Livestock, and Food Supply (MAPA) were used. The program comprises a great number of different feed ingredients. Samples were analysed using an in house validated method based on EN 16215:2012¹, PLE on ASE 350 (Thermo), clean up on Dextech Heat, followed by quantification by GC-HRMS (Autospec, Waters) or GC-MS/MS (7010b, Agilent). Data from 2017 to 2022 were collected. For positive samples, congener concentrations were normalized in order to have the total concentration equal to 100 (%). Furthermore, data were organized according to the number of chlorines in the present congeners and congeners type (PDDD; PCDF or PCBs).

Results: The most critical feed categories in terms of contamination were: feed materials of animal origin - land animals (I); feed additives of sedimentary origin (II); vegetable oils and their by-products (III); fish meal (IV). It was possible to differentiate the congener profile for some categories. Group I presented low chlorinated PCDD and PCDF, both contributing equally to the total TEQ, being PCDFs formed in higher concentrations. This pattern seems to be related to burning/drying process² used for processing this kind of material. Group II were characterized by the presence of low chlorinated dioxins (PCDD) only. This compounds could be present in the raw clay². Groups III and IV can be separated from the others by the presence of PCBs mainly. It is not clear if the presence of PCBs in fish meal and vegetable oil came from a contaminated raw material or due to an improper processing.

Discussion and conclusion: The congener profile characterization is a good starting point to be used in investigations of PCDD/F or PCBs contamination incidents in food. Biotransformation factors, bioconcentration factors and transfer rates to the animal product were not studied, however must be also considered.

Acknowledgements
The authors would like to thank the CGAL/SDA/MAPA for the support.

References:
Introduction: Perfluorocarboxylic acids (PFCAs) have a structure consisting of a hydrophobic perfluoroalkyl group and a hydrophilic carboxylic acid. These compounds are characterized by an extremely high chemical stability, resulting in persistence in the environment. Then, these are bioaccumulated and cause adverse effects to health in humans and wildlife. As one of enzymes that metabolize pollutants, such as polychlorinated biphenyls (PCBs), cytochrome P450 (P450 or CYP) monooxygenases from various organisms have been identified. P450BM3, which is obtained from the soil bacterium Bacillus megaterium, intrinsically hydroxylates long-chain fatty acids (C12-C20) and shows the highest activities among P450s because it contains a reductase domain in one molecule. To date, many P450BM3 mutants that become to show catalytic activities toward non-original substrates have been produced. Furthermore, by the addition of decoy molecules, which have similar structures to long-chain fatty acids but not metabolized, P450BM3 becomes to show hydroxylation activities to non-original substrates. As decoy molecules, PFCAs were used because of its metabolic initiation ability and high chemical stability. Using this reaction system, the hydroxylation of 2,3',4,4',5-pentachlorobiphenyl (CB118) by P450BM3 wild type (WT) was strongly enhanced. In contrast, it is possible that PFCAs are reacted by P450BM3 since P450BM3 binds to PFCAs. In this study, we will show the possibility of PFCA reactions by P450BM3 WT and its mutants.

Materials and Methods: We added PFCAs with the carbon chain length from 6 to 14 (C6–C14) as a substrate and recombinant P450BM3 WT and seven P450 BM3 mutants to the reaction mixture containing NADPH as an electron donor. The consumption rate of NADPH and the production amount of H2O2 were measured. Docking models of P450BM3 and PFCA were constructed to investigate the metabolic potential of P450BM3.

Results: The consumption rate of NADPH increased as the carbon number of PFCAs increased. This may be due to the fact that the longer the carbon chain, the closer the terminal part of PFCA is to heme, the reaction center of P450BM3. This result is supported by the calculation of the binding free energy and the distance between the carbon of PFCAs and heme iron based on the docking models. Furthermore, among these P450BM3, WT and three mutants were selected based on their high NADPH consumption rates and the change in rates with carbon number of PFCAs. The metabolic potential was inferred from the low H2O2 production and the difference with NADPH consumption.

Discussion and Conclusion: Our results suggest possible reactions of PFCAs by P450BM3. Since PFCAs are chemically stable compounds, degradation of PFCAs leads to environmental remediation. In the future studies, degradation products should be revealed.

Acknowledgments: This study was, in part, supported by The Iwatani Naoji Foundation.

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Metabolism

P-071  Metabolism of 2,2',4,4',6,6'-Hexachlorobiphenyl by Rat, Guinea Pig and Human Liver Microsomes and Human Cytochrome P450

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Introduction: PCBs, the well-known worldwide environmental pollutants, consist of 209 homologues or isomers, depending on the number of chlorine atoms and their substitution positions. Among them, PCBs having 2,4,5-trichloro- or 2,3,4,5-tetrachloro-substituted benzene such as 2,2',4,4',5,5'-hexaCB (PCB153), 2,2',3,4,4',5'-hexaCB (PCB138) and 2,2',3,4,4',5,5'-heptaCB (PCB180) are known to be highly persistent and have been detected in high concentrations in human blood and tissues1-4. On the other hand, PCBs having 2,4,6-trichloro-substituted benzene (hereinafter abbreviated as 246-type PCB) including PCB154, PCB182 and PCB188 are rarely detected in human tissues. So far, we demonstrated that 246-type PCBs are hydrolyzed more easily than 245-type ones in rats5,6. Therefore, we studied the metabolism of another 246-type PCB, 2,2',4,4',6,6'-hexachlorobiphenyl (PCB155), using rat, guinea pig and human liver microsomes, and human cytochrome P450 (CYP) isoforms. Three kinds of liver microsomes from untreated, phenobarbital (PB)-treated and 3-methylcholanthrene (MC)-treated rats and guinea pigs were also used.

Materials and Methods: PCB155 and its metabolite were synthesized by the method of Cadogan. Liver microsomes from male Wistar rats (body weight about 200 g) and Hartley guinea pigs (body weight about 300 g) were prepared the next day after the last ip injection of CYP inducers, PB and MC, at a dose of 80 and 20 mg/kg/day for three days, respectively. Human liver microsomes and CYP isoforms (CYP2A6, 2B6, 2C8 and 3A4) was purchased from Corning Inc. PCB155 (40 µM) was incubated at 37ºC for 60 min with 0.33 mM NADPH-generating system, 6 mM MgCl₂, 100 mM HEPES buffer (pH 7.4) and 1 mg protein of rat or guinea pig liver microsomes in a total volume of 1 ml (0.5 ml in human enzymes). After incubation, unchanged CB155 and its metabolite were extracted three times with the mixture of 1 ml of chloroform-methanol (2:1, v/v) and 3 ml of n-hexane. The organic layer was pooled and evaporated to dryness. The residue was methylated with diazomethane and applied to GC-ECD and GC-MS.

Results: In rats, PCB155 was metabolized to M1 by PB-microsomes at a high rate of 4.55 nmol/hr/mg protein, but not by both untreated microsomes and MC-microsomes. In guinea pigs, both untreated microsomes and MC-microsomes showed the activity at rates of 0.06 and 0.06 nmol/hr/mg protein, respectively, whereas PB-microsomes increased M1 to 3.5-fold of untreated microsomes (0.19 nmol/hr/mg protein). In contrast, human liver microsomes also produced only M1 and the activity was 0.29 nmol/hr/mg protein. GC-MS analysis demonstrated that the methylated M1 had the molecular weight of 388 and almost completely agreed with a synthesized authentic 3-methoxy-PCB155 about the retention times and mass fragmentation. Of four human CYP isoforms, only CYP2B6 showed high activity to form M1 (0.70 nmol/hr/nmol CYP).

Conclusion: Consistently in rats, guinea pigs and humans, the major metabolite M1 was 3-hydroxy-PCB155. The fact that PB treatment increased M1 suggests the involvement of PB-inducible CYP2B enzymes. In agreement with PCB153 metabolism7, CYP2B6 plays the most important role in PCB155 metabolism in human liver.

Acknowledgments: This work was supported by a Health and Labour Scientific Research Grants (21KA2003, C.O.) from Ministry of Health, Labour and Welfare of Japan.

References:
Micro(nano)plastics as Environmental Vectors for POPs and Additives

P-073 Source and Pathway of Microplastics in Groundwater from Japan

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Introduction:
Groundwater is an important resource of available freshwater supplying with 2 billion people in the world1). However, little information is available on the status of microplastics (MPs) pollution in groundwater, although an increasing number of MPs have been detected in the aquatic environment. Our preliminary study reported the occurrence and distribution of MPs (>100 mm) in groundwater collected from Kumamoto, Japan2). The mean abundance of MPs was 1.1 items/100 L, and polyethylene (PE) was a dominant polymer, but little was known on their seasonal variation, source and pathway in groundwater. Based on the background, groundwater samples were collected monthly for 1.5 years and analyzed for MPs to understand the temporal trend. In addition, organic additives in MPs were identified by Py-GC/MS and the profiles was compared with those in commercial plastic products to estimate the potential source. Further, MPs in dropped water in caves was analyzed to investigate the possible pathway of MPs from soil surface to groundwater.

Materials and Methods:
Groundwater samples were monthly collected in Kumamoto, Japan during July 2021 and November 2022. One hundred litters of groundwater were filtered on site with 100 µm-mesh nylon filter, and the filtrates were treated by 30% H2O2 for degradation of organic materials. MPs candidates were collected and the polymer type was identified by FT-IR. In groundwater and dropped water in cave collected from Okinawa, 1 L of sample was filtered with 10 µm-mesh nylon filter to investigate the detection profiles of MPs at the size of 10-100 mm by µFT-IR.

Results and Discussion:
MPs were detected in groundwater samples from Kumamoto, Japan (33% to total), and the average was 0.76±1.8 items/100 L. PE was dominant polymer and blue and transparent were major colors of MPs. In seasonal variation of MPs, the abundance increased in August rainy season, and then decreased in autumn, October to November (Fig. 1). However, the abundance increased again during December to February, while the amount of precipitation was relatively low in winter. These results imply that rainwater play an important role to transport MPs from the soil surface to aquifer layers, but another pathway may be present of MPs migration into groundwater. The results of Py-GC/MS in MPs and commercial plastic products suggested that agriculture materials may be one of potential source of MPs in groundwater.

MPs including <100 mm were analyzed in groundwater from Okinawa. MPs were detected in more than 90% of the samples analyzed, indicating the ubiquitous distribution and contamination in groundwater. The median was 52 items/L, which was 3 orders of magnitude greater than the value of >100 mm MPs in groundwater. PE was major polymer (56%), followed by polyester (PES) and polypropylene (PP). Interestingly, groundwater samples in Okinawa had a high abundance of fibrous MPs, which implies the sewer leakage into groundwater.

MPs were detected in dropped water in caves at Okinawa. PE and PES fragments and fibers were dominant, which were almost similar to those in groundwater. The upper surface of the cave was situated by road, apartment and livestock farm, suggesting that rainwater may have carried MPs from the surface to the groundwater.

Reference:
2. Okita and Nakata (2023) Abstract of 58th Annual Conference of JSWE.

Acknowledgments: We would like to thank to all staffs cooperating groundwater sampling. This study is partly supported by SATREPS in collaboration between JST (Project#: JPMJSA1901) and the Environmental Research and Technology Development Fund by the Ministry of the Environment, Japan (Project #: JPMEERF21S11900).
**Introduction:** Microplastics (MPs) are ubiquitous plastic debris that may pose a risk to the food chain via interaction with organic contaminants (OCs). MPs undergo physical, chemical, and biological aging in the environment, which may in turn affect their interaction with OCs. Although OCs typically co-exist with aged MPs in aquatic ecosystems, studies related to the adsorptive role of especially bio-aged MPs are quite limited. This study aims to investigate the bio-aging potential of low-density polyethylene (LDPE) and its effect on sorption of three model OCs; namely, malachite green (MG), triclosan (TCS), and 2,3,6-trichlorophenol (TCP).

**Materials and Methods:** Bio-aging of LDPE was accomplished in mesophilic (i) anaerobic digesters (BMPs), and (ii) aerobic reactors containing waste activated sludge (WAS). LDPE of 425-500 µm particle size was dosed at 300 mg/g TS in BMPs and 5 g/L in WAS reactors. Biogas volumes and methane content were monitored in BMPs, while BOD5 reduction was measured in WAS reactors. Upon termination (60th day), sludge parameters were analyzed, MPs recovered and characterized. Then MPs were washed with H2O2 and characterized via FTIR, SEM, and DSC. Sorption of OCs investigated using pristine, H2O2 washed and bio-aged+H2O2 washed MPs at 9ppm Cinitial, 10 g/L S/L, pH=4, 6, and unadjusted for TCP, TCS, MG, respectively, to test unionized forms, in 40 mL vials shaken at 200 rpm at 25oC±2oC. OCs analyzed spectrophotometrically at appropriate wavelengths.

**Results and discussion:** Anaerobic and aerobic reactors did not show any sign of inhibition from the high dose of MPs. VS reduction was 38.7% and 42.7%, while BOD5 reduction was 98.8% and 96.3%, for aerobic and anaerobic reactors, respectively. Also, test and control BMPs had similar biogas and methane yields. MPs from both reactors showed signs of bio-aging, as observed from FTIR spectra (Figure 1). Apart from common LDPE backbone peaks, new bands are observed for aerobic and anaerobic-aged samples. The new bands observed at around 3300 cm⁻¹ are attributed to the stretching vibrations of O-H. The new bands at 1653, 1645, and 1545, 1542 cm⁻¹ indicate protein-based materials. The broadband with increasing intensity at 1082 cm⁻¹ indicates polysaccharides, which are significant components of microbial biofilms (Bonhomme et al. 2023). Also, the band at 1735 cm⁻¹, seen only for aerobic-aging LDPE samples, shows C=O ester stretching vibrations. The carbonyl index of all aged samples is as zero according to SAUB method. However, for aerobic-aging LDPE, the CI is 0.03 using A1735/A1470 method. Such changes due to bio-aging may affect sorption onto MPs favorably or unfavorably. Currently, sorption experiments with pristine LDPE indicate an affinity order of TCS>TCP>MG, where MG being highly soluble, showing very low affinity. Our previous work on the sorption of TCS and TCP with bio-aged HDPE showed an unchanging affinity for TCS yet a complete loss of affinity for TCP (Dit Tahan et al., 2023). Hence, similar comparative sorption testing will be conducted to investigate changing sorption tendencies of model OCs with bio-aged LDPE potentially showing oxidation, peeling, etc.

**Conclusions:** Concurrent bio-aging of LDPE-type MPs in anaerobic and aerobic reactors, with an extensive characterization of resulting MPs, will bring valuable information on the impact of two different types of biological activities on the aging of a common MP type in the environment. Furthermore, comparative testing of sorption of three physico-chemically diverse model OCs on bio-aged LDPEs enable exploration of change of sorption mechanisms and interaction between OCs and environmentally relevant MPs.

**Acknowledgments:** This study is supported by TUBITAK Project No: 220N044

**References:**

Micro(nano)plastics as Environmental Vectors for POPs and Additives

P-075 Interaction of an ionizable halogenated organic with high and low density polyethylene

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Introduction: Microplastics (MPs) became ubiquitous components of the environment, negatively affecting human health and ecosystems. The potential vector effect as well as the toxicity of MPs and organic contaminants (OC) require scrutinizing MP-OC interactions. There are relatively smaller number of studies for ionizable OCs. For this purpose, 2,3,6-trichlorophenol (TCP) was chosen as a model compound to investigate interactions as neutral and ionized forms onto commonly found high-density (HDPE) and low-density polyethylene (LDPE).

Materials and Methods: Amber vials (40 mL) were used in triplicate, together with controls (TCP only and PE only) as sorption reactors. MPs of 250-500 µm particle size at an S/L ratio of 25 g/L were shaken at 200 rpm at 25°C±2°C using deionized water. Considering the pKa of TCP as 5.9, the pH of sorption reactors was set at pH=4, 6±1 (unadjusted) and pH=8. Kinetic experiments yielded teq as 24hrs and 4 hrs for HDPE and LDPE, respectively. Isotherm experiments were conducted at 7 different concentrations for both polymer types and at pHs 4 and 6.

Results: TCP has much lower affinity for LDPE when compared to HDPE. Sorption capacity was distinctly increased when unionized TCP dominated the system for both PE types. The sorption capacity for LDPE was 135µg/g at pH 4 and 98µg/g at pH 6. The same trend was observed for HDPE, with higher sorption capacities; 291 µg/g at pH 4 when compared to 228 µg/g at pH 6. Isotherm plots for HDPE and LDPE at different pHs were fitted to both Langmuir and Freundlich models (R² >0.97). A sample result from sorption studies is shown in Figure 1. As can be seen, the completely ionized form of TCP at pH 8 had much lower affinity for either type of polymer, when compared to the unionized or partially ionized form.

Figure 1: Change of sorption capacity of 2,3,6-TCP at three different pH values.

Discussion and Conclusions: Sorption capacity increasing with the unionized form of TCP dominating in the system was also observed by Liu et al. (2020), where when the pH of the solution reached 10, the dissociated TCP yielded nearly negative Kd values. Our zeta potential measurements show pHPZC of both polymers to be less than 2, indicating a negative charge on the microplastic surface at the pH values tested here. As a result, substantial electrostatic repulsion between the negatively charged PE and negatively charged phenolate would be expected, which would prevent their adsorptive interaction at pH values close to and above the pKa of 5.9. Furthermore, the affinity of TCP was observed to be consistently higher for HDPE when compared to LDPE. The two polymers differ from each other, especially in terms of their crystallinity (49.5% for HDPE and 25% for LDPE). Our results indicate that the interaction of TCP with crystalline portions of the polymer, as opposed to the amorphous regions, have a positive impact on sorptive capacity. The mobility and perhaps bioavailability of chlorinated phenols may increase when sorbed onto MPs. For those having a pKa close to ambient pH values, sorptive behavior and interaction with microplastics is expected to be impacted by the pH of the surrounding matrix, potentially harming aquatic life and the ecosystem.

Acknowledgments: This study was funded by TUBITAK Grant No: 220N044.

Micro(nano)plastics as Environmental Vectors for POPs and Additives

P-076 Plastic-City: European cities as sources for microplastics and associated organic contaminants to their waterways

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Introduction: Plastics, when discarded to the environment and transformed to micro plastics (MP), are persistent and have a high potential to cause physical and toxicological harm. Large knowledge gaps have been identified in two domains: (1) origin, abundance, and distribution of microplastics in freshwater systems and (2) toxicological and environmental effects (JPI Oceans, 2020). Among the various sources of MPs identified for freshwater, cities with their high population density and plastic-based domestic activities, are hotspots for MP deliveries towards aquatic systems (Kataoka et al. 2019). This work presents the research strategy and very first results of the Plastic-City research project (09.2023 – 08.2025). The project focuses on the city of Brussels, as a typical European city and aims at (1) quantifying the losses of MP towards its waterways (river Zenne and a navigation canal) in relation to domestic waste and wastewater management practices and (2) determine the associated interaction/transport of specific persistent organic micropolllutants such as endocrine disrupting chemicals (eg. xenoestrogens) and polyaromatic hydrocarbons (PAHs), their alkylated homologues Me-PAHs, PCBs, and pesticides.

Materials and Methods: The research strategy and methodology follows 3 main axes. The first concerns the assessment of the urban plastic cycle: quantities collected in domestic waste and recycled, lost to the combined sewers, retained at wastewater treatment plant (WWTP) inlets, transferred as MPs to the treatment process and finally delivered to waterways. In the second, we identify and quantify selected toxic organic compounds in the environment and wastewater, and their association to MPs. Finally, in the third, we determine their toxicity using bioanalytical screening techniques. Here the research results of the first 9 months are presented: (1) a first estimation of the macro plastics in untreated wastewater; (2) the optimization of 2 sampling-enumeration-analyses methods for MPs and first results; (3) the optimization of water sample pre-treatment for GCMS analyses and quantification tests for a standard cocktail of PAHs, Me-PAHs and PCBs; (4) the establishment of an estrogenic substances (ES) budget in the Brussels river Zenne based of previously collected data.

Results: (1) Waste samples collected at the inlet of a Brussels WWTP showed a plastic content of 45g/kg waste and high MPs concentrations. (2) The 2 MP sampling methods (volume reduced method for MP>300 µm and bulk sampling method for MP>10 µm) show consistent results with the highest concentrations in sewage and lowest in river and canal waters, but absolute numbers are very different (MPs>10µm = 100 x MPs>300µm). MP concentrations and daily fluxes in the river increase from up to downstream the city. (3) The GCMS pretreatment method was optimized. It is highly sensitive, with a detection down to 5 pg for the tested contaminants in both Full scan and SIM mode. The Full scan mode allowed collection of spectra for all injected compounds, enabling the creation of a customized library for identification purposes (4) The total daily load of ES emitted by the 2 Brussels WWTP were between 0.44 and 1.64 g E2 eq./d and are the most important source for the river (31%). The second important source are combined sewer overflows (CSO) (27%).

Discussion and Conclusion: First results show that: (1) there is an important loss of plastics to the sewers of the city; (2) the untreated sewage is enriched in MPs compared to river and canal water, but also in ES. Any CSO towards surface waters will thus result in important contaminations; (3) The 2 MP sampling methods concern different fractions (>10µm and >300µm) and give different results, but are complementary and in line with other studies (Koelmans et al 2020); (4) Brussels is a sources of MPs and ES for its waterways; (5) The optimized pretreatment method and highly sensitive GCMS analysis will be used for all samples to detect and quantify the PAHs, Me-PAHs and PCBs in water samples; (6) Collected MPs>300 µm are retrieved after analyses for contaminant adsorption/desorption experiments to be performed; (7) ES in the Zenne River behave in a pseudo-persistent manner because of continuous input from the WWTPs and slow degradation. The bio-equivalent concentration of E2 exceeds the EU EQS of 0.4 ng E2/L everywhere in the Zenne River.

Acknowledgments: Plastic-City is supported by a PRFB-Innoviris grant.

Micro(nano)plastics as Environmental Vectors for POPs and Additives

P-077 Size-resolved identification and quantification of micro/nano-plastics in indoor air using pyrolysis gas chromatography-cyclic ion mobility mass spectrometry

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Introduction: Humans are likely exposed to micro-/nanoplastics (MNPs) through inhalation¹, but few studies have attempted to measure airborne MNPs²,³. This might be attributed, in part, to a paucity of analytical techniques that can identify and quantify MNPs without constraints related to their size⁴. This presentation reports on the development of a novel (non)targeted screening (NTS) approach to identify and quantify MNPs in the indoor environment using pyrolysis gas chromatographic cyclic multiplexed with ion mobility mass spectrometry (pyr-GCxcIMS).

Materials and Methods: The cIMS enables the simultaneous measurement of m/z, collision cross section (CCS), and RT for all pyrolysis decomposition products as well as plastics additives sampled through thermal desorption. The method was applied to size-resolved particulate (56 nm-18µm) collected from two different indoor environments, viz. a laboratory space and a private residence. A variety of common plastic types were targeted, including polystyrene (PS), polyethylene (PE), polypropylene (PP), and polymethyl methacrylate (PMMA).

Results: The results suggest that approximately 57-67% of airborne MNPs are characterized by particle diameters less than 2.5 µm. The NTS experiments also revealed the presence organophosphate esters whose abundance correlated with that of polyurethane (PU) (r = 0.85, p<0.05), which is consistent with their use as flame retardants in PU-based furniture and construction materials.

Discussion and Conclusion: The results of our study provide insight into the concentrations of MNPs in the indoor environment. The mean concentrations of MNP particles with diameters <2.5 µm in the two sampling locations, ranging from 16 – 27 µg/m³, exceed short term (24-hour) and long-term exposure guidelines suggested by the World Health Organization. This result, and the fact that approximately 50-60% of the PM2.5 present in the private residence are MNPs, raises underline the critical need for further study of this route of exposure to MNPs and the (unknown) plastics additives carried with them.

Acknowledgments: The authors gratefully acknowledge support from the Natural Sciences and Research Council (NSERC) and the Government of Canada’s New Frontiers in Research Fund (NFRF).

References:
Per and Polyfluorinated Substances (PFAS): Occurrence and Exposure

P-078  Investigation of legacy and emerging PFAS in wastewater samples through target and suspect screening: an unclosed mass balance

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Introduction: Wastewaters can represent a continuous source of PFAS in the environment, and in addition to legacy PFAS, new alternatives and emerging PFAS may pose additional risks as conventional treatment plants are unable to remove these recalcitrant and persistent compounds. Moreover, the presence of precursors, i.e., substances that have the potential to be transformed into perfluoroalkyl acids (e.g., PFOA and PFOS), can lead to further risks. In fact, even if perfluoroalkyl acids are regulated and/or banned, we still see an increase in the environmental pollution over time resulting from precursors' degradation. Investigating these substances in wastewater samples (influent and effluent) is important mainly for two reasons: 1) wastewater is a complex matrix and various wastewater treatment processes can lead to the degradation of precursors; 2) the release of wastewater effluents directly into receiving water bodies can have a major impact on the aquatic environment. However, the assessment of this group of heterogeneous compounds requires an analytical effort that cannot be limited to a target analysis, which is why a suspect screening was carried out in parallel with the target analysis, with the aim of fully characterizing the PFAS contamination profile in the influent and effluent wastewater.

Materials and Methods: Influent and effluent samples were collected for five consecutive days at a Flemish WWTP (Belgium) in August 2022. The 24h-composite samples were extracted by solid phase extraction using WAX cartridges for target analysis and HLB and WAX cartridges for suspect screening. Liquid chromatography-tandem mass spectrometry (LC-MSMS) was used for the quantification of 27 target compounds [1] and LC-quadrupole-time-of-flight MS (LC-QTOF-MS) for the suspect screening [2]. For the latter, two suspect lists (> 10,000 PFAS) were used to match the m/z features of suspect compounds that were ranked according to exact mass and isotopic pattern. Compounds with the highest scores were re-analysed with selection of relevant precursor ions to obtain the MS2 fragmentation and increase the confidence level (CL) up to 2 when matched with experimental and or in silico spectra [3]. A semi-quantification of the CL2 suspect compounds and high-scoring CL4 compounds was done to estimate the concentration of the identified compounds in the wastewater samples.

Results: The results of the target analysis showed that in the influents only PFHxA was detected above the limit of quantification (LOQ), and the concentrations ranged from <LOQ to 55 ng/L. In the effluents, PFPeA, PFHxA, PFOA, PFDA and PFOS were detected in a total concentration range of 188 to 301 ng/L and PFDA was the most predominant. The suspect screening results highlighted the presence of some emerging PFAS identified with CL2, such as 6:2 FTAB (trade name: Capstone B) and PFOSI (CAS n° 647-29-0), an environmental transformation product of precursors. For CL2 and high-scoring CL4 suspect compounds (n=12), semi-quantification showed similar concentrations between influents and effluents, with maximum concentration never exceeding 300 ng/L.

Discussion and Conclusion: Comparing the results of this study with a previous investigation of PFAS in the influent of the same WWTP, the concentrations are highly comparable [2]. In general, this low level of contamination is probably due to the limited contribution of industrial discharges. The concentrations of the target compounds were higher in the effluents compared to the influents, resulting in a negative removal efficiency. One explanation can be that the degradation of precursors during the wastewater treatment contributes to relatively higher concentrations of perfluoroalkyl acids in the effluents. Starting from that, the expectation was to identify a higher proportion of precursors in the influents, but the concentrations of the semi-quantified compounds in the influents and effluents were similar. A further step might be the investigation of suspect compounds in other fractions of materials in the WWTP (e.g., sludge) but the low overall contamination made the suspect screening very challenging. In fact, this approach is a useful but also very time-consuming tool: a tier approach based on total methods as preliminary investigation - such as the Total Oxidizable Precursor Assay (TOPA) - might help prioritize the most contaminated samples worth investigating using suspect and/or non-target analysis.

References:
Per and Polyfluorinated Substances (PFAS): Occurrence and Exposure

P-079 Development of analytical method for PFAS in rise using QuEChERS extraction and LC-MS/MS

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Introduction
Per- and polyfluoroalkyl substances (PFAS) are widely used in industrial and consumer products, and their persistent and bio-accumulative characteristics raise concerns about human health. To assess PFAS exposure and potential risks through dietary intake, it is essential to develop efficient and sensitive analytical methods for detecting PFAS in food samples. Currently, the US EPA and FDA have announced methods applicable to rise. The EU has proposed regulations for PFOS, PFOA, PFHxS, and PFNA, and recommended limit of detection (LOD) for targeted foods. In this study, we compared two methods for 24 types of PFAS (PFBA, PFPeA, PFHxS, PFHpA, PFOS, PFNA, PFDA, PFUnA, PFDoA, PFTrDA, PFTeDA, PFBS, PFPeS, PFHxS, PFHpS, PFOS, PFNS, PFDS, N-EtFOSAA, N-MeFOSAA, PFOSA, 4:2FTS, 6:2FTS, 8:2FTS). We developed and validated a method for analyzing PFAS in rise using LC-MS/MS after comparing purification efficiency and cost-effective pretreatment methods.

Materials and Methods
FDA-based modified method: 5 g of homogenized white rice was placed in a 50 mL conical tube. We added an internal standard material (Extraction STD) and 5 mL of water, then mixed them for 1 minute using a vortex. After that, 10 mL of acetonitrile and 150 µL of formic acid were added, and the sample was treated with an ultrasonic assisted extraction for 30 minutes. QuEChERS Salt was added, followed by 2 minutes of vortexing, 20 minutes of shaking, and centrifugation at 10000 rcf for 10 minutes. The supernatant was added to the d-SPE sorbent and shaken for 10 minutes, then centrifuged at 10000 rcf for 10 minutes. The upper layer (4 mL) was separated, and to avoid complete drying, 100 µL of water was added before drying. We then added 90 µL of methanol and 10 µL of Syringe STD to reconstitute the test solution. After filtering through a 0.2 µm syringe filter (nylon), the sample was analyzed by UHPLC LC-MS/MS (AB SCI-X).

EPA-based modified method: The sample was extracted in an alcoholic KOH solution for 16 hours at room temperature. After the d-SPE purification step in the FDA-based modified method, the pH was adjusted and processed through a Weak anion exchange Cartridge, then concentrated by drying with high purity nitrogen.

Methanol, acetonitrile, and water of LC grade were purchased from Merck. QuEChERS extraction salt (4 g of MgSO4 and 1 g of NaCl) and d-SPE sorbent (900 mg MgSO4, 300 mg PSA, 150mg Graphitized carbon black) were purchased from Restek. We used Waters’ Oasis WAX (6 cc, 150mg Sorbent, 30µm Particle Size) for the Weak anion exchange Cartridge. PFAS standard materials (PFAC-MXH, MXG, MXF, MXI, MXJ), internal standard material (MPFAC-HIF-ES), and syringe added standard material (MPFAC-HIF-IS) were purchased from Wellington Laboratories (Guelph, Ontario, Canada).

Results
There was virtually no difference in recovery rates between the two methods. In this study, we developed and validated the simpler FDA-based method. The results showed good linearity (R²>0.99), precision (relative standard deviation < 15%), accuracy (recovery rate 70-120%), detection and quantification limits of 0.1-0.5 ng/g and 0.3-1.5 ng/g, respectively.

Discussion and Conclusion
While the EPA-based method required a longer extraction time than the FDA method, the increased number of preprocessing steps resulted in lower recovery rates, making the difference in recovery rates between the two methods negligible. In this study, we developed a method for analyzing PFAS in grains using QuEChERS extraction and LC-MS/MS.

Acknowledgments
This study was supported by the Ministry of Food and Drug Safety under Grant [22161MFDS004] in 2023.
**Introduction:** Per- and polyfluoroalkyl substances (PFASs) are anthropogenic chemicals developed in the 1940s. Especially, perfluorosulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) were in past use worldwide as plasticizers, coatings, fire-extinguisher foams and so on. PFASs have been investigated with respect to human exposure and risk assessment from environmental contamination, because they are reported to cause cancer, reproductive damage, and immunological deterioration. Therefore, the international legal regulations of PFASs are being widely discussed, and the Stockholm Convention on Persistent Organic Pollutants (POPs) has decided to add perfluorohexanesulfonic acid (PFHxS) to Annex A (substances whose manufacture, use, import, and export should be prohibited) in addition to PFOS and PFOA. The assessment of PFASs levels in Japanese food and drinking water is required for safety assurance. Indeed, in 2020, the Japanese Ministry of Health Labour and Welfare established a provisional target value (50 ng/L) of sum PFOS and PFOA in tap water. However, studies of PFASs in bottled water are very limited in Japan and the need to regulate PFASs in bottled water has not been discussed yet. Here, we developed a simultaneous analytical method for twenty PFASs in bottled water by LC-MS/MS.

**Materials and Methods:** Twenty PFASs consisting of nine perfluoroalkyl carboxylic acids, nine perfluoroalkyl sulfonic acids, DONA and F-53B were purchased on the commercial marker. The solid-phase extraction (SPE) cleanup and concentration for bottled water was performed with a Presep PFC-II cartridge (60 mg/3 mL, FUJIFILM Wako Pure Chemical Co). The LC-MS/MS system was a Waters ACQUITY UPLC I-Class /Xevo TQD (Waters Co.). Reversed-phase chromatography analysis was performed using an InertSustain C18 column (2.0 x 100 mm, HP series, 3 µm, GL Science Inc.) at 40°C. The mobile phase, consisting of solvent A (2.5 mmol/L ammonium acetate in water) and solvent B (acetonitrile) was delivered at flow rate of 0.3 mL/min. The injection volume was 5 µL. The delay column for PFASs (GL Science Inc.) was used in the LC system, and the blank water sample and experimental water were filtered using an activated carbon filter (InertSep Slim-J AC, GL Science Inc.) to suppress background contamination from the analytical procedure.

**Results:** In this study, the mobile phase and analytical column of LC system were improved from the previous study1. As a result, an InertSustain C18 column was selected for PFAS separation. In addition, the optimal concentration of ammonium acetate was investigated (2.5–10 mmol/L), and sufficient sensitivity was achieved at 2.5 mmol/L. The previous study1 selected methanol as the solvent B, but it was not able to separate PFASs which have the same carbon numbers. The Separation (resolution >1.5) including branched-chain PFASs was observed in the mobile phase using acetonitrile. The lower limit of quantitation (LOQ) was determined to be 2.5 ng/L for twenty PFASs in blank bottled water. For two concentration (5 ng/L and 50 ng/L), the trueness and within-laboratory reproducibility were found to be 84–105% and 0.7–12.0% (n=2, 5 days), respectively. To verify the practical application of the analytical method, Japanese and foreign bottled water samples were analyzed by the analytical method. The recovery and repeatability for two concentration levels met the Guidance Document on Analytical Parameters for the Determination of Per- and Polyfluoroalkyl Substances (PFAS) in Food and Feed (recovery 65–135%, within-laboratory reproducibility 25%). Our method can contribute to the investigation of PFASs in bottled water in Japan.

**Discussion and Conclusion:** In this study, a LC-MS/MS method was developed for twenty PFASs including PFOA, PFNA PFHxS and PFOS. After optimization of the mobile phase and analytical columns, the adequate peak shapes of PFASs were obtained within 20 min using this analysis. In addition, the background in the measurement procedure was decreased to 1/5 of LOQ. The recovery and repeatability for two concentration levels met the Guidance Document on Analytical Parameters for the Determination of Per- and Polyfluoroalkyl Substances (PFAS) in Food and Feed (recovery 65–135%, within-laboratory reproducibility 25%). Our method can contribute to the investigation of PFASs in bottled water in Japan.

**References:**
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1. Introduction:
Per- and polyfluoroalkyl substances (PFAS) have been widely used in industries as a group of emerging persistent organic pollutants. PFAS can persistently remain in water, soil, even in food, and seem to have potential threat to human health. Rice (*Oryza sativa* L.), the most essential staple food crop in Asia, is currently consumed worldwide. However, there is a lack of knowledge on PFAS in rice and information on material balance inventories in paddy fields. To address this issue, it is necessary to accurately assess contaminants such as PFAS in rice and its cultivation environment. This study examined the distribution of 32 PFAS in air particulate matter (PM), cultivated soil, irrigation water, rainwater, and two varieties of rice (Indica and Japonica rice). In addition, fluxes of PFAS were evaluated based on concentrations in the cultivated environment matrices.

2. Materials and Methods:
Sample collection

*Rice.* Rice samples were cultivated in a paddy field (10 m × 50 m) at a National Agriculture and Food Research Organization (NARO) agricultural experiment station (36°1′ 28.6″ N, 140°6′ 25.0″ E) in Tsukuba, Japan from May 2020 to September 2020. The Indica variety, "Kasalath" and Japonica variety, "Koshihikari" were selected. Each variety of Indica and Japonica rice was planted in half of the same paddy (5 m × 50 m). Irrigation water was kept by continuous flooding during cultivation according to general practices of rice cultivation in Japan. The total cultivation periods of Indica and Japonica in the paddy field were 114 and 123 days, respectively. After harvesting, rice samples were washed with ultra-pure water and then oven-dried at 70 °C for one week. The rice samples were segregated into ear, root, and stems, including leaves at 0–20 cm, 20–40 cm, 40–60 cm, and > 60 cm. The rice ear was separated into husk, bran, and white rice. A total of 8 parts of rice samples were prepared.

*Soil.* Pre- and post-harvest soil were collected in the paddy field. The soil samples contained a pool of 17 subsamples which were collected from the cultivated horizon (0–15 cm). After collection, soil samples were passed through a 5 mm mesh sieve and air-dried for one week.

*Irrigation water.* Irrigation water was collected directly from the water outlet and kept in PP bottles.

*Rainwater.* Rainwater was sampled by a PP funnel connected with a 1 L PP bottle at the rooftop of the building near the agricultural experiment station during the cultivation period. The rainwater samples were collected for 5 rainfall events.

*Atmospheric particulate matter (PM).* PM samples were collected by using a PM sampler (Type-NS20; SIBATA Scientific Technology Ltd., Tokyo, Japan) with pre-baked quartz fiber filters (QFFs). The PM sampler was deployed at the same location with the rainwater sampler during the cultivation period. Details of sampling system and sampling procedure were followed those established in the previous studies1, 2.

*Extraction and instrumental analysis*
All samples were spiked with mass-labelled PFAS prior to extraction. Solid samples (rice, soil, and QFFs) were extracted by solid-liquid extraction with acetonitrile for rice or methanol for soil and QFFs. After extraction, concentrated extracts were purified by SupelcleanTM Envi-CarbTM (100 mg, 1 mL; Sigma-Aldrich Corp., St. Louis, MO, USA) cartridges and Oasis® weak anion exchange (WAX; 6 cc, 150 mg, 30 μm; Waters Corp., Milford, MA, USA) cartridges. The detailed information of extraction procedure was given in the previous reports3–6.
Thirty-two ionic PFAS target analytes (C4, C6–C8, and C10 PFSAs, C4–C14, C16, and C18 PFCAs, FOSA, N-MeFOSA, N-EtFOSA, N-MeFOSAA, N-Et FOSAA, 6:2FTSA, 8:2FTSA, 8:2FTUCA, 10:2FTUCA, 8:2diPAP, 6:2Cl-PFESA, HFPO-DA, DONA) were analyzed using an Agilent 1260 Infinity liquid chromatograph (Agilent Technologies, Santa Clara, CA, USA) coupled with a Triple Quad 4500 mass spectrometer (AB Sciex, Framingham, MA, USA). The mean recoveries of mass-labelled standards were within a range of 60–103% in rice, 64–102% in soil, 52–115% in irrigation water, 61–112% in rainwater, 72–103% in PM. The PFAS concentrations reported in this study were not corrected for mass-labeled standard recoveries.

3. Results and Discussion:

PFAS concentrations in the agricultural environment and rice

Figure 1: Average PFAS concentrations (pg/g dw for soil and rice, ng/L for irrigation water and rainwater, and pg/m3 for PM).

Figure 1 shows the concentration of individual PFAS in the agricultural environment, including soil, irrigation water, rainwater, PM, and several parts of rice.

The average concentrations of the total PFAS (Σ32PFAS) collected before and after cultivation was 2392 pg/g dw. There were no significant differences before and after cultivation. PFOS, PFNA, and PFUnDA were the prominent compounds in soil. C4–C12 PFCAs were always detected in the PM during cultivation period, whereas shorter chain (< C8) PFSAs were not detected. 6.2FTUCA, PFNA, and PFHxA were dominant compounds in PM with concentrations of 1.8, 1.7, and 1.6 pg/m3, respectively.
Σ32PFAS in rainwater ranged 8.9–36 ng/L. Differed with air samples, rainwater samples contained PFOS (0.26 ng/L) of PFSAs, as well as the short-chain compounds PFBS (0.09 ng/L) and PFHxS (0.22 ng/L). The dominance of shorter chain PFAS was observed. PFHxA, PFPeA, and PFOA were the predominant PFAS in irrigation water. This was consistent with the results of rainwater. Compared with the ratio of PFSAs and PFCAs in air and water, more PFSAs were remained in the cultivated soil.

The average concentration of Σ32PFAS between Indica and Japonica rice was 631 pg/g dw in the root, 500–1695 pg/g dw in the stem, and 56–526 pg/g dw in the ear including the husk, bran, and white rice, respectively. In the edible portion of white rice, average concentration of Σ32PFAS between Indica and Japonica was 56 pg/g which was the lowest concentration among the rice plant. Both PFOS and PFOA in Indica were 3 pg/g dw, whereas those in Japonica were 3 pg/g dw and 4 pg/g dw, respectively. In this study, no clear differences of PFAS concentration in the rice varieties were observed.

Calculation of PFAS flux in the agricultural environment

To help complete understanding of PFAS mass balance inventories in the agricultural environment, total masses and fluxes of PFAS were estimated. The masses of Σ32PFAS in soil and rice were estimated by multiplying the concentration of Σ32PFAS in soil or rice by the bulk density of soil or the mass of rice plant per one square meter, respectively. The amount of rice plant per one square meter is generally 1500 g/m² in Japan7.

The flux of Σ32PFAS through irrigation water was derived by multiplying the concentration of Σ32PFAS in irrigation water by the daily volume of irrigation water applied to the paddy field which is estimated from the lysimeter experiment in Japan reported elsewhere4.

The flux of Σ32PFAS through wet deposition was calculated by multiplication of Σ32PFAS concentration in rainwater by the volume of precipitation during the sampling period divided by multiplication of surface area of the PP funnel used for rainwater sampling by the number of sampling days of rainwater. The flux of Σ32PFAS through dry deposition was estimated by multiplication of Σ32PFAS concentration in PM by the deposition velocity of PM, which is generally ranged 0.1–1 cm/sec 8.

The estimated PFAS flux in paddy field from one cultivation period was summarized in Figure 2.

Figure 2: Estimated Σ32PFAS flux in paddy field from one cultivation period.

4. Conclusions:
The current study focused on the residue distribution of PFAS and the estimation of total PFAS mass and flux in the paddy field environment. The flux of irrigation water was the largest contribution to the paddy field. Considering that rice is cultivated mostly (50–60 days) in a water-soaked environment, occurrence of PFAS in irrigation water may be more important for the PFAS residue in rice than the other crops. To our best knowledge, this is the first time that estimation of PFAS flux in the agricultural environment. Further studies are needed to understand the PFAS accumulation pathways in agricultural products.
5. Acknowledgments:
This work was supported by JSPS KAKENHI (Grant Numbers 20J01877, 22H02486, 21H04949, and 20KK0245) and the Environment Research and Technology Development Fund of the Environmental Restoration and Conservation Agency of Japan (1G2102). The author would like to thank Dr. Tadashi Abe for his helpful advice on various technical aspects of rice cultivation in this study.

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1. Introduction:
Per- and polyfluoralkyl substances (PFASs) have been widely used in industrial and commercial products, such as surfactants, cosmetics, and food contact materials. But now there is an urgent need to regulate or eliminate their production and use due to concerns about their bioaccumulation and toxicity. Under the Stockholm Convention on Persistent Organic Pollutants, perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid (PFOS), and perfluorohexane sulfonic acid (PFHxS) were listed for elimination or restriction in their production and use. More recently, POPs Review Committee is also considering listing long-chain perfluorocarboxylic acids (C9–C21 PFCAs). To manage and evaluate the risks of these PFASs and related compounds including precursors, risk profiling based on environmental monitoring data is essentially important.

In Japan, guideline values for PFOA and PFOS have been set for water pollution prevention, and local governments have been required measurement of these PFASs in public water. While water quality regulations are progressing, air emissions of PFASs are not currently regulated under the Japanese air pollution control law. It has been suggested that PFASs are transferred into the atmosphere through the manufacturing process of products containing PFASs and incineration of fluoropolymers \cite{1}, as well as through oxidation of precursors in the atmosphere \cite{2}. A PFASs contamination in the downwind area of a chemical plant \cite{3} has awakened us to the importance of understanding the dynamics of atmospheric PFASs. The Japanese Ministry of the Environment has monitored only PFOA and PFOS (PFHxS was added in 2020) in the air using a conventional high-volume (HV) air sampler since 2010 \cite{4}. For a more comprehensive understanding of the environmental pollution of PFASs, the determination of atmospheric concentrations and phase distributions of many PFASs including substitute substances and precursors is necessary.

Our group developed a novel atmospheric PFASs sampler and an activated charcoal fiber (GAIACTM) that enabled us to quantify about 50 ionic and neutral PFASs in particle and gas phases. This technique had applied to air sampling in suburban areas in Japan \cite{5}, indoor and outdoor air \cite{6}, and open ocean \cite{7} and compared to the conventional HV sampler method \cite{8}. Currently, we have been conducting a nationwide survey of atmospheric PFASs in Japan for risk profiling. In this study, we report the results of measurements of ionic PFASs (including some neutral PFASs) in five regions of Japan in 2022.

2. Materials and Methods:
\textbf{1) Reagents and Materials}
Native and labeled stock solutions of PFASs mixtures for ISO 21675 were purchased from Wellington laboratories (Guelph, Ontario, Canada). Native PFASs mixture contained 13 PFCAs (C4–C14, C16, C18: PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA, PFHxS, PFOS), 5 perfluoroalkyl sulfonic acids (C4, C6–C9 PFSAs: PFBS, PFHxS, PFHpS, PFOS, PFDS), 3 perfluorooctane sulfonamides (FOSAs: FOSA, N-MeFOSA, N-EtFOSA), 2 perfluorooctane sulfonamidocarboxylic acids (FOSAAs: N-MeFOSAA, N-EtFOSAA), 2 fluorotelomer sulfonic acids (FTSAs: 6.2 FTSA, 8.2 FTSA), 8 fluorotelomer unsaturated carboxylic acid (8.2 FTUCA), and 4 other PFASs (9Cl-PF3ONS, 8:2 diPAP, HFPO-DA, DONA). The labeled PFAS mixture and 13C4-PFOS (Wellington laboratories) were used as surrogate and syringe spikes, respectively. Extraction solvent (special grade methanol, ethyl acetate, dichloromethane, acetone, and toluene) and mobile phase for liquid chromatography (LC-MS/MS grade methanol and water) were obtained from Daisanest (Tokyo, Japan). Polyurethane foam (PUF; 47 mm dia., 50 mm thick; Sibata Scientific Technology, Tokyo, Japan), and GAIACTM (47 mm dia., 2 mm thick; Futamura Chemical, Gifu, Japan) were used for air sampling media. Prior to sampling, QFF was prebaked at 350°C for 3h, and PUF and GAIACTM were cleaned up with water, methanol, ethyl acetate, and dichloromethane, successively.

\textbf{2) Air sampling}
Atmospheric PFASs were collected using an FM4 air sampler (GL Science, Tokyo, Japan) that is comprised of a four-stage cascade impactor followed by a PUF and GAIACTM holder (Figure 1). Size segregated particulate PFASs (>10 µm, 2.5–10 µm, 1.0–2.5 µm, <1.0 µm) were collected on QFFs, and gaseous PFASs were adsorbed on a PUF and two GAIACTM. Air sampling was conducted at a flow rate of 20 L/min for 48 h at Tokyo, Aichi, Osaka, Hyogo, Fukuoka in Japan (Figure 2) in 2022. Meteorological conditions during sampling are shown in Table 1 in brief.
Per and Polyfluorinated Substances (PFAS): Occurrence and Exposure

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Table 1: Meteorological conditions during sampling.

<table>
<thead>
<tr>
<th>Region</th>
<th>#</th>
<th>Date</th>
<th>Temp (°C)*</th>
<th>Rainfall (mm)</th>
</tr>
</thead>
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<tr>
<td>Tokyo</td>
<td>1</td>
<td>5/30</td>
<td>16.8-26.2 (20.1)</td>
<td>19.0</td>
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<tr>
<td></td>
<td>2</td>
<td>6/1</td>
<td>17.9-27.3 (22.2)</td>
<td>0.5</td>
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<tr>
<td></td>
<td>3</td>
<td>6/8</td>
<td>16.0-24.2 (19.2)</td>
<td>-</td>
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<tr>
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<td>6/8</td>
<td>18.1-27.4 (22.8)</td>
<td>-</td>
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<tr>
<td></td>
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<td>16.4-26.5 (19.4)</td>
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<td>6/15</td>
<td>18.6-30.5 (21.9)</td>
<td>1.5</td>
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<tr>
<td></td>
<td>2</td>
<td>5/27</td>
<td>18.4-30.8 (23.6)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>6/1</td>
<td>17.8-28.7 (23.3)</td>
<td>-</td>
</tr>
<tr>
<td>Hyogo</td>
<td>1</td>
<td>5/18</td>
<td>17.2-26.1 (21.3)</td>
<td>-</td>
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<tr>
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<td>2</td>
<td>5/21</td>
<td>16.9-23.8 (20.5)</td>
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<tr>
<td></td>
<td>3</td>
<td>5/23</td>
<td>18.6-26.2 (22.6)</td>
<td>-</td>
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<tr>
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<td>6/22</td>
<td>25.0-31.3 (27.7)</td>
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<td>2</td>
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<td>26.2-33.7 (29.3)</td>
<td>-</td>
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<tr>
<td></td>
<td>3</td>
<td>6/29</td>
<td>25.4-34.6 (29.7)</td>
<td>-</td>
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</table>

3) Sample treatment

Extraction and concentration methods were based on the previous report [6] with some modifications. Briefly, a surrogate solution was spiked to sampling media before extraction. QFF was extracted by methanol three times. PUF and GAIAC were extracted by ethyl acetate/dichloromethane (1:1, v/v) and methanol with 0.01% ammonia successively. These extracts were concentrated under gentle nitrogen stream and added a syringe spike solution and methanol. The methanol solutions were injected into the liquid chromatography-tandem mass spectrometer (LC-MS/MS) for measurement of the target PFASs.

4) Instrumental analysis

LC-MS/MS analysis was conducted by an ExionLC liquid chromatograph coupled with a Triple Quad 4500 tandem mass spectrometer (AB Sciex, Foster City, CA, USA). Conditions of LC-MS/MS analysis were referred to ISO 21675 and briefly listed as follows: analytical column, Betasil C18 (2.1×50 mm, 5 µm; Thermo Scientific, Waltham, MA, USA); guard column, Eclipse XDB C8 (2.1×12.5 mm, 5 µm; Agilent Technologies, Santa Clara, CA, USA); retention gap column, delay column for PFAS (2.0×30 mm; GL Science, Tokyo, Japan); mobile phase, 2 mM ammonium acetate and methanol; column temperature, 30°C; injection volume, 5 µL; flow rate, 0.22 mL/min; ionization, ESI negative; acquisition mode, multiple reaction monitoring; source temperature, 450°C. Before injection, 5 µL of sample solution was mixed with 10 µL of 2 mM ammonium acetate using autosampler.

3. Results:

1) Atmospheric concentrations of PFASs in five regions of Japan

Method detection limits of the targeted PFASs, determined from the lowest concentration of calibration solution, were 0.03–0.09 pg/m³. Recovery rates of surrogate compounds except for some compounds from QFF, PUF, and GAIAC were 48.3–87.4% (mean, 70.3%), 40.5–122% (86.5%), and 57.5–115% (87.5%), respectively. The labeled 13C2-6:2 FTSA and 13C2-8:2 FTSA were recovered 73.9–161% (108%) and 70.7–200% (110%) from sampling media. The recovery rate of 13C3-HFPO-DA (8.2–77.8% (42.2%)) was relatively lower than other PFASs.

We detected 19–24 ionic PFASs, namely 13 PFCAs, 5 PFSAs, FOSA, N-MeFOSA, N-EtFOSA, N-MeFOSAA, N-EtFOSAA, 6:2 FTSA, 8:2 FTSA, 9Cl-PF3ONS, 8:2 FTUCA, HFPO-DA, and DONA from the samples collected in five regions of Japan. Figure 3 shows the atmospheric concentrations and compositions of the PFASs. The sum of concentrations of particle and gas phase ionic PFASs was 130–610 pg/m³ and the predominant PFASs were PFBA, PFHxA, and PFBS. The total concentrations of PFCAs and FPSAs were 81–420 pg/m³ and 33–150 pg/m³, respectively. The mean total concentrations of ionic PFASs in Osaka was 490 pg/m³ and relatively higher than that of other regions (Tokyo; 210 pg/m³, Aichi; 180 pg/m³, Hyogo; 320 pg/m³, Fukuoka; 190 pg/m³). The concentration of the long-chain PFCAs (C9–C16) in Osaka was 44–95 pg/m³ and also higher than those in other regions (1.9–38 pg/m³). The differences in the PFASs composition were observed among samples from the same region (Figure 3 (b)). For example, the compositions of HFPO-DA (also known as GenX) in Tokyo, 6.2 FTSA in Aichi, and PFPeA in Hyogo differed by a factor of 3 to 4.
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2) Gas-particle partitioning of the PFASs

The particle size and phase distributions of the major PFASs in Osaka (mean, n=3) are shown in Figure 4 (a). As shown in this figure, the PFASs were almost detected from PUF and activated charcoal filters (GAIACTM), except for 6:2 FTSA. The 6:2 FTSA was only detected from QFF in Osaka.

Figure 4 (b) shows the mean gas-particle partition coefficients of the PFASs in five regions (n=3). The gas-particle partitioning of the PFASs was described using particle-associated fraction which was defined as a ratio of the PFAS concentration associated with the particle phase over the sum of gas and particle phases concentrations. The particle-associated fractions of PFCAs increased with increase in the length of carbon chain from 4 (PFBA) to 9 (PFNA). However, the particle-associated fractions of PFDA (C10, 0.049 ± 0.052) and PFDoDA (C12, 0.061 ± 0.065) were lower than those of PFNA (C9, 0.41 ± 0.31) and PFUnDA (C11, 0.31 ± 0.33).
4. Discussion:

1) Atmospheric concentrations of PFASs in five regions of Japan

We have constructed a database of PFASs in the atmosphere and river water in Japan for risk profiling. In this study, we measured the concentrations and phase distributions of 30 ionic PFASs in the atmosphere in five regions of Japan. The sum of the concentrations of ionic PFASs (130–610 pg/m³) in our study was relatively similar to those in suburban and urban areas in Japan (220–680 pg/m³, excluding C2 and C3 PFASs) reported by a previous study [6]. In addition, the concentrations of PFOA (3.8–24 pg/m³), PFOS (1.9–5.4 pg/m³), and PFHxS (1.7–5.5 pg/m³) in our study were within the ranges of the monitoring results in 37 sampling sites in Japan in 2020 [4] (PFOA: 4.9–55 pg/m³, PFOS: 1.1–7.2 pg/m³, PFHxS: 0.7–6.1 pg/m³).

Although the ionic PFASs concentrations of Osaka were relatively higher than those of other regions, the sum of the concentrations of ionic and neutral PFASs [9] of these regions were similar (640–1130 pg/m³), except for Fukuoka (330–470 pg/m³) (Figure 5). The relatively low PFASs concentrations in Fukuoka might be due to the sampling conditions and location (relatively high temperatures and suburban location). The characteristic concentration of some PFASs, such as long-chain PFCAs, HFPO-DA, 6:2 FTSA, and PFPeA, in each area indicates the variability of a source of atmospheric PFASs.

2) Gas-particle partitioning of the PFASs

The gas-particle partitioning of PFASs was an important parameter to predict its environmental fate. The particle associated fractions of PFASs in this study were relatively lower than those reported by previous studies [6, 10]. Ahrens et al. [10] measured 7 PFAS classes using an annular diffusion denuder sampler and reported that the particle associated fractions of PFCAs were increasing with fatty acid chain length (e.g., PFOA: 0.06, PFNA: 0.14, PFDA: 0.14, PFUnDA: 0.36, PFDoDA 0.28). The higher particle associated fractions of PFCAs in their study may be resulted from lower ambient temperature during their sampling periods (−9.4 to +7.7 °C). Interestingly, our result that the PFDoDA has a lower particle associated fraction than PFUnDA contrary to their vapor pressure was similar to their observation. It might be possible that precursors of PFCAs, such as fluorotelomer alcohols (FTOHs) [11], were adsorbed to PUF or activated charcoal fiber and then oxidized to PFCAs during the sampling period.

5. Conclusions:

We observed the 30 ionic PFASs in the atmosphere in 5 regions of Japan using a newly developed PFASs sampler (FM4) and absorbent (GAIACTM). The differences in the concentrations and compositions of ionic PFASs among samples suggested the variety of sources and dynamics of the atmospheric PFASs. We should analyze samples collected in other seasons for detailed profiling of the atmospheric PFASs.

6. Acknowledgments:

This research was performed by the Environment Research and Technology Development Fund (JPMEERF20211G02) of the Environmental Restoration and Conservation Agency of Japan.

7. References:


Per and Polyfluorinated Substances (PFAS): Occurrence and Exposure

P-082  Atmospheric concentrations and phase distributions of ionic per- and polyfluoroalkyl substances (PFAS) in five regions of Japan


Per and Polyfluorinated Substances (PFAS): Occurrence and Exposure

P-083 Occurrence of PFASs in Spanish wastewater treatment plants

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Unit of POPs and Emerging Pollutants in the Environment, Department of Environment, CIEMAT, Av. Complutense 40, 28040 Madrid, Spain

1 Introduction
Rivers can receive the input of treated or untreated effluents from wastewater treatment plants (WWTPs), urban and industrial discharges and agricultural run-off, becoming an important pathway for the transport and mobilization of pollutants to aquatic ecosystems. A kind of emerging organic contaminants of recent and increased concern involves the perfluoroalkyl substances (PFASs) which present persistence, toxicity, potential for bioaccumulation and remarkable ubiquity in the environment. In fact, perfluorooctanoate acid (PFOS), perfluorooctanoic acid (PFOA) and perfluorohexane sulfonic acid (PFHxS) have been included as persistent organic pollutants in the Stockholm Convention1, and long-chain PFCAs with carbon chain lengths from 9 to 21 are presently proposed for listing2. Furthermore, PFOS is also listed as substance of priority concern in the European Water FrameWork Directive3 due to its persistence, toxicity and widespread use and detection in rivers, lakes, transitional and coastal waters.

WWTPs are currently not equipped to efficiently remove PFASs from wastewater. PFASs are very recalcitrant and resistant to biological treatment, and as a result, can end up in the effluent or in sewage sludge. Moreover, some of them, such as PFOS and PFOA, could be generated during the biological treatment process in the WWTP from the degradation of polyfluoroalkyl precursor compounds4,5. Several studies have identified WWTPs as significant source of PFASs to the environment through effluent discharge to surface water, land application of biosolids and disposal of residuals or air emissions6, nevertheless data on concentrations in urban WWTP influents, effluents and sludge is scarce7.

In the present study, the distribution of 26 PFASs have been evaluated seasonally in the influent and effluent wastewater and sludge samples collected before and after the anaerobic digestion process from two Spanish wastewater treatment plants.

2 Materials and Methods
A total of 32 samples (16 wastewaters and 16 sewage sludges) from two wastewater treatment plants (WWTP1 and WWTP2) were collected during 2022 in four different monitoring campaigns (winter, spring, summer and autumn) to determine the presence of 26 PFASs, consisting of 4 perfluoroalkyl sulfonic acids (PFSAs; C4, C6, C8, C10), 13 perfluoroalkyl carboxylates (PFCAs; C4-C14, C16, C18), 3 perfluoroalkyl sulfonamides (PFOSAs; C8), ADONA and HFPODA (GenX). The WWTP1 was a conventional urban facility with biological (activated sludge) treatment while WWTP2 was equipped with advanced biological treatment with nutrient removal.

Wastewater samples (0.5 L) spiked with isotopically labelled surrogate standards (MPFAC-C-ES, M8FOSA, N-d3-MeFOSA, N-d5-EtFOSA and M3HFPO-DA solutions, Wellington Laboratories Inc.,Guelph, Canada) were loaded onto Oasis HLB (500 mg, 6 mL; Waters) cartridges. Previously, these were conditioned sequentially with 10 mL methanol and 10 mL ultrapure water. The entire sample was passed through the cartridge, dried under vacuum for 1 hour and eluted with 10 mL of methanol. Sludge samples (5 g) were also spiked with labelled standards and treated using the method described by Navarro et al., (2011)8. Briefly, samples were extracted in methanol by agitation during 10 min, sonication for 30 min and centrifugation. Then, a purification step was conducted using Oasis WAX (500 mg, 6 mL; Waters) and EnviCarb (500 mg, 6 mL; Merck). The final extracts were spiked with MPFAC-C-IS solution (Wellington Laboratories Inc.,Guelph, Canada) and analyzed on UHPLC-MS/MS (ExionLC Shimadzu-SCIEX Triple Quad 3500).

The identification and quantification were carried out using isotopic dilution method if proper standards were available. Procedural blanks were conducted and extracted under the same conditions than samples. In addition, instrumental blanks (methanol) were run before each sample injection to check the possibility of cross-contamination from UHPLC-MS/MS system.

3 Results and Discussion
PFASs were detected in all wastewater and sludge samples (Figure 1, Table 1 and 2). Eleven out of 26 compounds were found in wastewater (PFBA, PFPea, PFHxa, PFHpA, PFOA, PFNA, PFDA, PFUdA, PFDoA, PFBS and PFOS) while 15 were measured in sludge (PFBA, PFPea, PFHxa, PFHpA, PFOA, PFNA, PFDA, PFUdA, PFDoA, PFTtDA, PFTeDA, PFHxDA, PFBS, PFOS and FOSA). Sludges presented more number of compounds per sample (12, median) than wastewaters (8, median). Some pollutants such as PFDoA, PFPeS, PFHxs, PFHps, PFNS, PFDS, PFDoS, ADONA, HFPODA, N-EtFOSA and N-MeFOSA were not found in any sample. The observed distribution pattern of PFASs differed between the wastewater and sludge samples.

Wastewater samples
PFHxa, PFHpA, PFOA, PFNA and PFDA were the compounds most frequently detected (100%). The mean 11PFASs in wastewater was 33.5 ng/L (median: 35.8 ng/L; range: 6.53-144 ng/L) and the highest concentration measured (93.7 ng/L) was for PFBA in influent of WWTP1 followed by PFBS (56.6 ng/L) and PFHxA (20.6 ng/L). The high levels of PFBA and PFBS may reflect the
current use of these short-chain PFCAs and possible replacement of PFOA and PFOS.

Differences related to PFAS contribution was found in the two WWTP: PFBS (55%) > PFBA (47%) > PFHxA (16%) for WWTP1, and PFOA (28%) > PFBA and PFHxA (19%) for WWTP2. Nevertheless, similar PFAS levels were observed, 21.0 ng/L and 21.1 ng/L, median for WWTP1 and 2, respectively. The total concentration of PFASs in the influent was more elevated (50.1 ng/L, median) than the effluent (21.0 ng/L) in WWTP1 while the opposite behaviour was observed in WWTP2 (13.8 ng/L and 28.0 ng/L, median, influent and effluent) (Figure 1a). Specially, PFPeA, PFHxA, PFHpA, PFOA, PFNA, and PFDA two-fold increased between influent and final effluent. This tendency has been previously reported7,9-11 and can be explained by transformation pathways from precursors to stable PFASs12.

Regarding the different sampling campaigns, a slight decline was observed in ∼PFASs during summer (14.4 ng/L). The impact of temperature on PFAS biotransformation rates has not been studied in controlled wastewater microcosms13. However, warmer conditions facilitate biological activity in WWTPs, and summer temperatures have been shown to increase the removal of other compounds, such as pharmaceuticals14.

Sewage sludge samples
In this matrix, the 67% of the compounds showed detection frequencies above 80%. The mean 15PFASs in sludge was 14.3 ng/g d.w., dry weight, (median: 11.6 ng/g d.w.; range: 2.75-28.8 ng/g d.w.). PFDA presented the highest level (10.8 ng/g d.w.) followed by PFOS (5.98 ng/g d.w.). These findings are in accordance with several studies which evidences higher sorption capacity for longer PFCAs and PFOS compared to short chain compounds4,15.

Similar PFAS pattern was observed for the sludge from the two WWTPs, being PFDA (31%), PFDoA (17%) and PFOS (14%) the compounds with predominant contribution to the total. Nevertheless, WWTP2 (24.6 ng/g d.w., PFAS median) presented significant higher (p<0.01, U-Mann Whitney test) concentrations than WWTP1 (6.71 ng/g d.w.) (Figure 1b). Guerra et al, (2014) reported a notable PFAS formation during advanced biological nutrient removal treatment compared to other biological treatments11. This trend could be in accordance with the highest values measured in the WWTP2 sludges. Apart from that, no differences were registered before and after the anaerobic digestion process in the two WWTPs (6.54-6.97 and 21.0-22.8 ng/g d.w., before-after median, WWTP1 and 2).

In sewage sludge, a decrease in total PFAS levels were also appreciated in the summer sampling campaign (9.79 ng/g d.w.). Thus, it was hypothesized that PFASs would have a seasonal pattern caused by temperature, accelerating biotransformation during certain times of year.

4 Conclusions
The monitoring of PFASs in WWTP matrices reflects the current status in these facilities, pointing out the necessity of investigating in advanced and efficient treatments to contribute to the progressive reduction of emissions of hazardous substances to aquatic system and environment. Besides, the reported concentrations are of interest since the wastewater effluents reach rivers, and surface waters may be treated to generate tap water, where the presence of PFASs could have human health implications.

5 Acknowledgments
The present study is part of the project PID2019-105990RB-I00 founded by MCIN/AEI/10.13039/501100011033.

6 References
Per and Polyfluorinated Substances (PFAS): Occurrence and Exposure

P-083 Occurrence of PFASs in Spanish wastewater treatment plants


15. Filipovic, M., and Berger U., 2015. Are perfluoroalkyl acids in waste water treatment plant effluents the result of primary emissions from the technosphere or of environmental recirculation?. Chemosphere, 129, 74-80
Table 1. Concentration (ng/g d.w.) of PFASs in sewage sludge (before and after anaerobic digester) from WWTPs.

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<th>WWTP1</th>
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<td>Influent Winter N.D. 25.8 0.22 0.39 0.18 1.63 0.60 0.87 0.39 0.22 N.D. 3.50 0.83 0.07</td>
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Table 2. Concentration (ng/g d.w.) of PFASs in sewage sludge (before and after aerobic digester) from WWTPs.

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<th>Type</th>
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N.D.: not detected; PFfODA, PFfPeA, PFfHEA, PFfPhs, PFfBS, PFfOS, PFfADB, PFfFOS, PFfHfOS, PFfMeFOS; were not detected in any sample.
Perfluoroalkyl and polyfluoroalkyl substances (PFAS) have been widely used in energy, electronics, clothing, and pharmaceuticals fields due to their properties (hydro- and lipophilic, flame resistance, electrical insulation, dielectric characteristics, surface activity and chemical resistance). However, PFAS are highly persistent because they are rarely biodegradable, and their transport over long-distances raises concerns to the environment and ecology. In the Stockholm Convention (PoPs Convention), PFOA, its salts and related substances, and PFHxS, its salts and related substances are listed as Annex A (elimination) and PFOS, its salts, etc. as Annex B (restriction). While there have been many studies and developments of PFAS analytical methods in water samples, studies of PFAS analytical methods and contamination in soil samples in Japan and overseas have been conducted using various sampling and pretreatment methods because of the diverse and heterogeneous nature of soil samples. Hence, it’s difficult to compare and verify the data obtained from previous soil contamination surveys.

In order to examine the practicality of the tentative analytical method manual for 30 PFAS-related compounds in soil samples, an external accuracy evaluation test was conducted for a series of processes from pretreatment cleanup to data measurement and analysis using extraction solutions after soil sample collection. Some of the results are shared in this study.

**Materials and Methods:** Mixture of native PFAS solution (ISO21675-NSS) and labeled PFAS solution (ISO21675-LSS) (Wellington Laboratories) were used as standards. The soil extraction solutions were cleaned up in pretreatment firstly with Supelclean Envi-Carb SPE tube (Supelco) and followed by Oasis WAX (Waters). The calibration curve was prepared using a serial dilutions of the standard in methanol. The samples were analyzed in SRM mode with Waters ACQUITY UPLC H-Class PLUS/Xevo TQ-S micro mass spectrometer system. Water, 100 mM ammonium acetate and methanol were used as the mobile phase. Separation was conducted on a Waters ACQUITY UPLC BEH C18 column (2.1 × 100 mm, 1.7 µm), using a gradient of water (A) and methanol (C). 100mM ammonium acetate (B) was set at a constant value of 2%. Waters ACQUITY UPLC BEH C18 (3.0 × 50 mm, 1.7 µm) was used as an isolator column for retention gap purpose.

**Results and Discussion:** Recovery tests using test samples to which a known amount of ISO21675-NSS was added before cleanup showed that the 29 target compounds were recovered within 94-115% except for PFOcDA. The matrix effect in LC-MS/MS measurements was evaluated using test samples to which a known amount of PFAS standard solution was added to the cleaned-up analytical samples, and quantitative values within 100 ± 20% were obtained for all compounds. All compounds were detectable from 2 ng/L as the lower limit of the calibration curve, and 29 of them showed good results with correlation coefficients of r = 0.999 or higher in the range up to 20 µg/L. In this case, all target compounds were undetected in the solvent blank sample. In the soil extract solution used for the measurement, PFOS was detected at high concentrations, followed by PFHxS, PFHpS, FOSA, PFOA, and PFHxA. Reproducibility was extremely good, with %RSD of less than 10% for all compounds when each sample was measured at n=3. As for PFOcDA, there is concern about loss during pretreatment, and it is necessary to change the activated carbon cartridge used or to revise the composition ratio of water and methanol when injecting into Oasis WAX as a future consideration.

**Conclusion:** In the pretreatment cleanup, although PFOcDA remained an issue, it was verified that 29 compounds could be quantified with a good recovery rate. The matrix effect was also good and within 100±20%. As for quantitative analysis of PFAS in soil, 30 compounds could be detected from 2 ng/L. The quantitative values were reproducible with %RSD less than 10%.

**References:**
1. NARO DRAFT METHOD 202201: 2023, Determination of perfluoroalkyl and polyfluoroalkyl substances (PFAS) in soil
2. ISO 21675:2019 Water quality-Determination of perfluoroalkyl and polyfluoroalkyl substances (PFAS) in water-Method using solid phase extraction and liquid chromatography-tandem mass spectrometry (LC-MS/MS)
Per and Polyfluorinated Substances (PFAS): Occurrence and Exposure

P-085  The seventy-nine footprints of PFAS pollution in India - Nationwide distribution in road dust

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1. Introduction:
Per- and polyfluoroalkyl substances (PFAS) are an important group of anthropogenic emerging pollutants, which mainly comprise persistent ionic perfluorinated carboxylic acids (PFCAs) and perfluorinated sulfonic acids (PFSAs). PFAS refer to a group of fluorinated carbon-chain chemicals, known as nearly 4000 PFAS are used in the global market¹. The concern for these chemicals has increased due to their ubiquitous distribution in water column², air³, ⁴, and human blood⁵ as well as due to their bioaccumulative, persistent, and toxic characteristics⁶‒⁸. Although many studies have reported the occurrence of PFAS in various environmental matrices, information on the occurrence and distribution of PFAS in road dust is still limited, especially in developing countries like India. This study aimed to investigate nationwide levels of PFAS in seventy-nine road dust samples from India and their possible sources arising from urbanization and industrialization.

2. Materials and Methods:
Sample collection
The sampling location is shown in Figure 1. A total of 79 road dust samples were collected from 12 different states (i.e., Jammu & Kashmir, Uttar Pradesh, Bihar, West Bengal, Odisha, Andhra Pradesh, Tamil Nadu, Kerala, Maharashtra, Gujarat, Rajasthan, and Punjab) and the union territory (Delhi) in India between December 2017 and May 2018. Sampling locations in this study included various small rural villages and metropolitan cities at altitudes ranging from sea level to 5000 m above sea level. Road dust was collected from the roadside using stainless steel spatula. Samples were stored in polypropylene (PP) bags and immediately transported to the laboratory. Samples were stored at −20°C until extraction.

Sample extraction
Three grams of road dust sample was weighed in a PP tube and spiked with 1 ng of mass-labelled standards prior to the extraction. Samples were extracted with 4 mL of methanol in an ultrasonic bath for 20 min, followed by vortex mix for 30 sec. The extracts were centrifuged at 1000 rpm for 2 min and the supernatant was transferred to a new PP tube. This extraction procedure was repeated two more times to collect 12 mL of extract. The extracts were concentrated under nitrogen gas at 40°C to around 1 mL, and then sample extracts were applied to SupelcleanTM ENVI-CarbTM (100 mg, 1 mL; Supelco, Bellefonte, PA, USA) cartridges for clean-up. The cartridge was pre-conditioned using 3 mL of methanol, followed by 1 mL of sample extract loading. The eluate was mixed with 100 mL of Milli-Q® water (generated by Milli-Q® gradient system; Millipore Co., Bedford, MA, USA) and further purified using Oasis® WAX solid phase extraction cartridges (150 mg, 6 mL; Waters Corporation, Milford, USA). This clean-up process was described elsewhere⁹.

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Figure 1: Sampling location of road dust in India
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Instrumental analysis

Twenty-one PFAS were determined in this study, including C4 and C8 PFSAs, C4, C5, C7–C14, C16, and C18 PFCAs, two perfluoroalkyl sulfonamides (FASAs), N-ethyl perfluorooctane sulfonamidoacetic acid (N-EtFOSAA), two fluorotelomer sulfonic acids (FTSAs), 6:2 fluorotelomer unsaturated carboxylic acid (6:2 FTUCA), and 8:2 polyfluoroalkyl phosphoric acid diester (8:2 diPAP).

The samples were analyzed using LC-MS/MS, an Agilent 1260 Infinity liquid chromatograph (Agilent Technologies, Santa Clara, CA, USA) coupled with a Triple Quad 4500 mass spectrometer (AB Sciex, Framingham, MA, USA) in the electrospray negative ionization with multiple reaction monitoring mode.

The separation of target PFAS was performed using two analytical columns for confirmation using a BetaSil® C18 analytical column (2.1 mm i.d. × 50 mm length, 5 µm particle size; Thermo Fisher Scientific, Waltham, MA, USA) together with an ZORBAX Eclipse XDB-C8 guard column (12.5 × 2.1 mm, 5 µm; Agilent Technologies, Foster City, CA) and a RSpak JJ-50 2D ion exchange column (2.0 mm i.d. × 150 mm; 5 µm; Shodex, Showa Denko K.K., Kawasaki, Japan) with an OPTI-GUARD® anion exchange guard column (1 mm; Optimize Technologies, OR, USA). The injection volumes for BetaSil® C18 column and RSpak JJ-50 2D column were 5 µL and 10 µL, respectively.

Quality assurance and quality control (QA/QC)

All the samples were analyzed in duplicate, and results were not corrected by the recoveries of mass-labelled standards. Mean procedural recoveries (n = 16) ranged 64%–118% for native PFAS, except for N-EtFOSA and PFHxDA with a standard deviation (SD) of <30%. The recoveries of mass-labelled PFAS spiked onto samples prior to extraction ranged 60%–123% except for FOSAs.

Statistical analysis

Statistical analysis was performed using R version 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria) and Microsoft Excel 2016 (Microsoft Corporation, Redmond, WA, USA).

3. Results and Discussion:

Figure 2: Individual PFAS concentration (pg/g) in road dust of India. The horizontal line within the box indicates the median value, the boxes below and above indicate the 25th and 75th percentiles, respectively, and the whiskers below and above the box indicate the minimum and maximum values.

Figure 2 shows individual PFAS concentration (pg/g) in road dust of India. The sum of total concentrations of 21 PFAS (∑21PFAS) ranged 23.3–861 pg/g with a median concentration of 116 pg/g. PFOS was found predominant (1.58–496 pg/g) among PFSAs, followed by PFBS (3.30–98.4 pg/g). ∑PFSAs ranged 1.58–496 pg/g with a median concentration of 22.3 pg/g.
Per and Polyfluorinated Substances (PFAS): Occurrence and Exposure

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PFCAs such as C4−C18 (except C6) were observed predominant in road dust of India, with 0.33−117 pg/g concentrations. The median concentrations of C5−C18 PFCAs were found with decreasing trend (from 8.90 pg/g to 1.66 pg/g) with the increase in the carbon-chain length. Long-chain PFCAs (C7−C18) predominated (~60%) the PFAS composition in Jammu & Kashmir state in North India, which indicates the photodegradation of precursors or long-chain PFAS at high altitudes. The sampling locations in the Leh city at the Jammu & Kashmir state were at high altitudes ranging from 3000 to 5400 m. Leh is the cold arid region of India that receives the highest amount of radiation that is around 7−7.5 kWh m−2 day−1, which could possibly transform precursors to long-chain PFCAs. Previous studies have indicated the widespread occurrence of PFAS precursors in the Indian atmosphere.

Among three quantified FOSAs/AAs, N-EtFOSA (0.33−21.1 pg/g) predominated in samples compared to N-EtFOSAA (0.33−2.04 pg/g). In South India states, i.e., Andhra Pradesh and Kerala, N-EtFOSA was found predominant compared to other states of India. A similar phenomenon was observed that N-EtFOSA was predominant in the Chennai city of South India before. N-EtFOSA is known as an active ingredient of sulfluramid, which is a pesticide applied to control termites and other crawling insects. The predominance of N-EtFOSA in South India suggests there might be a higher agricultural use of sulfluramid in this region.

Correlation analysis was performed as shown in Figure 3. A significant correlation was observed between PFCAs and PFSAs ($r = 0.62; p = 1.5 \times 10^{-9}$). This suggests that these chemicals may share a common source across different microenvironments or may be transformed from similar precursors. Interestingly, PFSAs were observed to exhibit significant positive correlation with FTSAs which are the precursors of PFSAs ($r = 0.51; p = 6.2 \times 10^{-6}$).

Among precursors (FOSA & FOSAAs and FTSAs), higher concentrations (Figure 2) and a significant correlation were observed, indicating higher usage of these chemicals in the urban areas of India. Industrial activities and usage of more packaged food items could be possible reasons for the high concentrations of 8:2 diPAP in urban areas.

4. Conclusions:
In the present nationwide study, 21 PFAS were measured, including 2 PFSAs, 12 PFCAs, 2 FASAs, N-EtFOSAA, 2 FTSAs, 6:2 FTUCA, and 8:2 diPAP. PFAS were detected with varying concentrations in road dust samples collected from different cities of India. PFOS was the most abundant compound among all PFAS quantified. Levels of PFAS in urban road dust were higher compared to those in rural road dust, suggesting urbanization as a contributor to PFAS levels. To the best of our knowledge, this is the first study to report the concentrations of PFAS together with their precursors in nationwide road dust samples in India.

5. Acknowledgments:
We thank Prof. P.V.V. Prasada Rao (Andhra University, Visakhapatnam, India) and Mr. Nanda Gopal (National Institute of Ocean Technology, Chennai, India) for their help during nationwide sample collection. This work was funded by JSPS KAKENHI, Grant Numbers 20KK0245 and 21H04949; and the Environment Research and Technology Development Fund of the Environmental Restoration and Conservation Agency of Japan (10-2102).
Per and Polyfluorinated Substances (PFAS): Occurrence and Exposure

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6. References:
**Introduction:** Kinetic data on single per- and polyfluorinated alkyl substances (PFAS) in humans are scarce or missing, but very relevant for risk assessment. Some data were reported for people with high exposure and decline of internal exposure after stop of a high external exposure, however, these data may be imprecise due to ongoing low level exposure. We therefore investigated kinetics of 15 isolated, mostly isotope-labeled PFAS after single oral application.

**Materials and Methods:** The standard compounds 13C3-PFBA, 13C3-PFPeA, 13C2-PFHxA, 13C7-PFHpA, 13C4-PFOA, 13C5-PFNA, 13C9-PFDA, 13C2-PFUdA, 13C12-PFDoA, PFBS, 18O2-PFHxS, 13C4-PFOS, 6:2FTS ("H7PFOS"), HFPO-DA ("GenX"), and DONA were purchased from Wellington (Guelph, Canada) or Cambridge Isotope Laboratories (Tewksbury, MA, U.S.A.). The compounds (doses about 4 µg, but about 19 µg in case of PFBS and HFPO-DA, chosen because of their low mass spectrometric sensitivity) were dissolved in ethanol and pipetted on a muffin weighing 120 g. The muffin was eaten within 10 minutes by a volunteer (K. A., physician, body weight 82 kg). Serial EDTA blood samples were taken every 15 minutes during the first 2 hours, and progressively less frequent in the following hours, days and weeks. Plasma samples were spiked by specific amounts of differentially labeled PFAS which allowed quantification of the 15 applied compounds by UPLC-MS/MS after extraction using OASIS WAX columns. Furthermore, faeces were collected completely over the first 6 days after application. Fecal PFAS were analyzed after extraction with methanol and ENVI-Carb cartridges by UPLC-MS/MS using the same set of differentially labeled PFAS.

**Results:** PFAS in the faeces samples of the first 6 days revealed a more or less complete absorption (less than 1% of the dose applied found), but not for 6:2FTS and 13C12-PFDoA (about 2.5% of the dose applied found for each compound). Analysis of a selection of 10 plasma samples of the first 2 weeks (4 h, 12 h, 1, 2, 3, 4, 6, 9 and 14 days) revealed the shortest half-lives for the short-chain PFAS 13C3-PFPeA (about 0.5 d, decay from 325 ng/L after 4 h to 7 ng/L after 3 d), 13C2-PFHxA (about 1.4 d, decay from 370 ng/L after 4 h to 4 ng/L after 9 d), 13C3-PFBA (4.7 d) and PFBS (more than 40 days). The 3 "alternative" PFAS also revealed relatively short half-lives for HFPO-DA (about 2.7 d), 6:2FTS (about 4.8 d), and DONA (about 18 d). For the PFAS with longer chains, the time of observation was too short to calculate half-lives. With respect to volumes of distribution, data of the first 2 weeks revealed values between 75 and 150 mL/kg body weight for most compounds. However, the final values may be higher due to possible redistribution processes occurring in the first months after application. Data on half-lives, possible redistribution processes and volumes of distribution obtained after several months after application will be presented at Dioxin 2023.

**Discussion and Conclusion:** Data in general demonstrate excellent to good rates of intestinal absorption. As the 15 compounds were applied as isolated PFAS not embedded in a food matrix, absorption rates may be somewhat lower under real-life conditions. Half-lives observed after the first 2 weeks after application roughly reflect data reported by others. An exception may be the half-life of PFHxA (about 1.5 d), which was reported to be much longer (geomean 32 d, range 14 to 49 d) in 7 professional ski wax technicians (Russell et al., 2015). The very different half-lives observed in this and others studies are likely due to different renal reabsorption which is thought to be mediated by certain transporters with high binding of long-chain PFAS (e.g. Louisse et al., 2023). This investigation is a pilot study to be followed by a study with a higher number of participants to reveal the variability of PFAS kinetics in humans.

**References:**
Introduction:
PFAS (Per- and Polyfluoroalkyl Substances) is a group of compounds made by human for a variety of applications. Some of the studies have linked exposure of some of these PFAS compounds to adverse health effects for human and animals. The regularatory monitoring of PFAS has focused on targeted quantitative LC-MS/MS. However, these methods are limited in scope due to the need for reference standards. Unlike traditional targets where reference standards are available, less than 200 PFAS standards exist for the more than 12,000 known PFAS, which emphasizes the need for a non-targeted workflow.

This presentation describes the comprehensive integration of emerging and conventional PFAS analysis techniques into a singular Compound Discoverer™ software workflow. Built-in data reduction approaches, including fragmentation-based target filtering leveraging similarity searches via the mzCloud™ spectral library, Fluoromatch Suite database containing over 700 PFAS signature fragments, and molecular networking using a CF2 transformation to connect homologous series, will be demonstrated. These approaches circumvent the lack of authentic standard availability, sparse coverage in spectral libraries, and limitations with negative mode in-silico fragmentation, enabling MS2 matching of PFAS absent from spectral libraries. Additional data reduction tools, including extensive mass lists of known and theoretical PFAS, MD filtering thresholds specific to fluorine-containing compounds, CF2 Kendrick MD, and orthogonal MS1 PFAS discrimination plots, ensure the retention of only targets exhibiting PFAS characteristics. Finally, a molecular network generated from all the preserved targets is showcased by Perfluorosulfonic acid and Perflurosulfonamide clusters, encompassing homologous series constituents neglected by conventional techniques and unknowns.
Per and Polyfluorinated Substances (PFAS): Occurrence and Exposure

P-088 Analysis of PFAS Compounds in Wastewater with US EPA Method 1633 Using Semi-Automated Solid Phase Extraction

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Introduction: The United States Environmental Protection Agency (US EPA) has released multiple drafts of Method 1633 which will focus on testing for 40 native Per- and Polyfluoralkyl Substances (PFAS) compounds in biosolids, fish tissue, ground water, landfill leachate, soil, surface water, and wastewater (1). The list of PFAS compounds is more extensive than existing methods 533 and 537.1 and the new 1633 method can be used for a wide variety of matrices.

For water samples Solid Phase Extraction (SPE) has developed over the last few decades into a fast and reliable method to analyze water samples for a wide variety of chemicals. Many environmental laboratories have specifically a need for high throughput of water samples being analyzed for PFAS. This includes both drinking- and wastewater. A semi-automated technique for Solid Phase Extraction (SPE) of PFAS compounds in water was developed which is inexpensive and has little chance of mechanical breakdown, the only automated part being a vacuum pump. Some of the work that was done with this system on method 1633 is presented here.

Material and Methods for EPA 1633: Twelve synthetic wastewater samples (500 mL) were spiked with 25 ppt native PFAS standards and relevant internals. Sample bottles were loaded onto the system and rinse bottles were filled with 5 mL reagent water. Weak Anion Exchange (WAX) cartridges were installed in each of the twelve positions, the vacuum turned on, and conditioned with 15 mL of 1% methanolic ammonium hydroxide followed by 5 mL of 0.3M formic acid. Samples were loaded across the cartridges at 5-10 mL/min (~ 8-inch Hg). The sample bottles were then automatically rinsed with 5 mL reagent water (twice) followed by 5 mL of 1:1 0.1M formic acid/methanol and these rinses were loaded onto the cartridges. The cartridges were then dried under vacuum for 15 seconds. Rinse bottles were filled with 5 mL of 1% methanolic ammonium hydroxide used to rinse the empty sample jars. The rinses were then loaded across the cartridges. Extracts were collected in polypropylene tubes and as per the method no further concentration was carried out. Relevant standards were added prior to LC/MS analysis.

Results: A total of 40 native PFAS compounds were analyzed using EPA method 1633. All recoveries were 90% or higher, except for FPDoS (~ 74%) and 11Cl-PF3OudS (~ 82%). RSDs (%) were all < 9%. The highest recovery found was for PFHxS at 103%. All PFAS recoveries were within the acceptance windows (different for each compound) required by the method.

An important problem with ground and wastewater extraction is the presence of particulate matter which can easily plug up cartridges. Use of plastic filtration wool in the barrel of the cartridges can eliminate this problem. In this work no clogging of cartridges was observed.

Conclusions: Wastewater samples can be analyzed with USEPA method 1633 using a simple laboratory semi-automated SPE set-up and use of plastic filtration wool. Recoveries were excellent with almost all values > 90%. RSDs observed were < 9 %. The system described here is an inexpensive alternative to fully automated SPE instrumentation.

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Introduction:
Per- and polyfluoroalkyl substances (PFAS) are a group of more than 5,000 synthetic organofluorine chemicals that were first developed in the 1940s and are still to this day used in many industries. The bioaccumulation of PFAS in our environment is an emerging topic, current scientific research suggests that exposure to high levels of certain PFAS may lead to adverse health outcomes. Two principal analytical methods in the US exist, EPA 533 (drinking water) & EPA 1633 (everything else). In various regions of the world regulations for PFAS in solid samples are already in place or being drafted.

EPA 1633 is currently a draft method which contains validation results for solids based on a single laboratory study for a total of 40 target PFAS across nine compound classes. We describe a comparable analytical LC/TQ method for soil samples with a sample preparation procedure which uses polymeric WAX SPE for enrichment followed by carbon matrix material for matrix clean up.

Materials and Methods:
5 grams of soil sample was weighed and prepared with spike matrix target and dilution analogue mix (EIS Extracted Internal Standard was added). For the extraction, 10mL ammonium hydroxide in methanol was added and centrifuged. 10g of carbon matrix was added, shaken and centrifuged. The sample was passed through SPE media of Weak Anion Exchange (WAX) sorbent for the extraction and recovery of PFAS compounds. After further filtration using a syringe filter the sample was analyzed using a binary HPLC which was adapted to reduce PFAS contamination. The LC system was coupled to a triple quadrupole equipment with an electrospray ion source. For data acquisition and analysis an appropriate MRM database was used at the optimal settings.

Results:
Initial precision and recovery and for this study, the overall average RSD was 3.8 ±0.6% (95% confidence level, 64 measurements) compared to the EPA draft method, the overall average RSD was 3.9±0.6% which give a confidence level of 95% which demonstrates efficient extraction method in order to achieve MDL's for each native PFAS at 99% according to EPA 1633 section 9.2.2. However, the calculated differences between MDLs in this study versus those in the published draft method show, except for PFBA, the smallest differences between the two data sets occur for the straight chain alkyl sulfonic and carboxylic acids, while the fluorotelomer sulfonic acids, fluorotelomer carboxylic acids, ether sulfonic acids, and sulfonamide ethanol’s show greater differences. This may indicate wider method optimization ranges for the typical alkyl carboxylic and sulfonic acids and narrower optimization ranges for PFAS with more complex alkyl moieties and functional groups. Matrix spikes for topsoil were used as an additional assessment of matrix effects, they can be used to assess matrix effects of native PFAS in which there is no isotope analogues. For both sample spikes, average recoveries were 97 ±1 1% which indicates the sample preparation steps offered an outstanding performance in matrix. For most compounds, the EIS recoveries for the topsoil extracts are within the minimum and maximum recoveries listed in Table 9 of the draft method.

Discussion and Conclusion:
The results of this application note demonstrate that the use method adopted utilizing PFAS WAX SPE and carbon matrix clean up provides comparative results to the US EPA draft method 1633 for the single lab validation study for solid matrices. Some variability was found amongst the results of four compounds during replicate sampling to measure precision. The greatest contributors to variability were attributed to sample inhomogeneity and sample mass differences. The topsoil contained pieces of twigs and small rocks that were difficult to remove, and samples masses were approximate, with recoveries scaled to a nominal 5 g dry mass which includes further variability.

References:
Per and Polyfluorinated Substances (PFAS): Occurrence and Exposure

P-090 Determination of Per and Polyfluoroalkyl substances (PFAS) in soils using a carbon matrix SPE by LC/MS/MS CMA/3/D

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European methods
CMA/3/D – Compendium for Sampling and Analysis (PFAS)
Used in Belgium and the Netherlands, developed by VITO

Introduction:
Per- and polyfluoroalkyl substances (PFAS) are man-made contaminants of critical concern due to their persistence and ability to bioaccumulate within the environment. One major focus of PFAS contamination is the analysis of contaminated land by sampling soil. Naturally soil is a difficult matrix to prepare for analysis due to its complex mixture of organic and inorganic compounds. Many of these organic compounds are co-extracted into the organic solvent along with the target analytes during sample preparation and cleanup. Without the further removal of these co-extractives, direct injection of extracts can result in multiple matrix effects upon analysis, and accumulation of matrix deposits in the sample flow path and MS ion source. We describe an approved European analytical method CMA/3/D which uses a cleanup step to remove matrix co-extractives prior to instrument analysis, without affecting the recovery of 59 PFAS target compounds from reed sedge peat and topsoil. These two matrix types were selected due to their very different physical and chemical properties.

Materials and Methods:
A sample of 2 grams of soil matrix was weighed for the sample preparation procedure. For the determination of recovery, accuracy, and precision, experiments were performed using two spiking levels of loamy sand matrix. The extract was then decanted into the carbon matrix SPE with advanced hybrid carbon material with optimized carbon content and pore structure. A 5µL injection volume of the extract is analyzed on a binary LC system that was adopted to eliminate the PFAS system background. Analysis was performed using a C18 column 2.1 x 100mm using a gradient elution of ammonium acetate in water and methanol. A triple quadrupole MS was used in negative ionization mode.

Results:
The concentration of PFAS residue measured in the topsoil were all below the MRL. PFAS levels exceeding the MRL were only found in the peat sample. The concentration of PFBA, PFPeA, and PFHpA measured in the peat sample were 4.51, 2.98, and 0.83 ng/g, respectively. The method performance was first evaluated by measuring recovery accuracy and precision of five replicate extractions at two spiking levels in the loam sand matrix. The recoveries reported were great for all compounds at 99.3% with an RSD of 13.5%. Reed sedge and topsoil were used for PFAS residue analysis. Extraction blanks were taken from duplicate cartridges of carbon matrix to ensure there was no PFAS contamination is being introduced during the sample preparation. The background concentrations of PFAS in the blanks were well below the 1/3 MRL threshold for all target PFAS confirming the low-level spike as the MRL. Significant pigment removal was achieved for both matrix extracts. For the peat, the extract color was orange/brown before Carbon S cleanup and became a barely perceptible yellow after passing through the sorbent. For topsoil, the extract was a slight yellow before cleanup and turned completely clear after cleanup. This shows efficient pigment removal was achieved for all samples. Chromatographic analysis before the cleanup using carbon matrix and after confirmed better peak shapes, reduce the matrix effects for some targets and improve data quality and consistency.

Discussion and Conclusion:
We conclude a robust and reliable passthrough matrix cleanup method for PFAS analysis in soil samples. For reed sedge peat extract, the use of the carbon matrix SPE cartridge improved the peak shape integrity and retention consistency of PFBA compared to extracts without the use of carbon matrix cleanup. These results demonstrate that the efficient matrix cleanliness provided by Carbon S passthrough cleanup can reduce the matrix effects for some targets and improve data quality and consistency. The results show that use of the carbon matrix SPE cartridge provided efficient passthrough matrix cleanup for PFAS analysis in soil samples.

References:
2. Giardina, M. Analysis of Per- and Polyfluoroalkyl Substances in Soil Extracts, Agilent Technologies application note, publication number 5994-2999EN, 2002
Per and Polyfluorinated Substances (PFAS): Occurrence and Exposure

P-091 Validation of an Extraction Methodology for the Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Whole Blood Employing Microsampling

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Introduction: Per- and polyfluoroalkyl substances (PFAS) have been present in the blood of greater than 99% of the general population for over 20 years (Olsen et al., 2003). Within this period, regulation has accelerated the development of alternative PFAS, with over six thousand on the NORMAN Suspect List Exchange. It is vital that the concentrations of these fluorinated compounds can be monitored within the human body given the World Health Organisation listed perfluorooctanoic acid (PFOA) as a potential carcinogen. PFAS blood analysis has been primarily undertaken through the analysis of PFAS serum content, however, some PFAS are more concentrated within whole blood. Therefore, increasing efforts have been made to establish robust methods for the analysis of PFAS in whole blood, commonly via dried blood spotting techniques (Poothong et al., 2019). This study aims to incorporate a commercial Microsampler (Trajan hemaPEN®) for the collection of dried blood spot (DBS) samples (sample volume – 10.96 µL ± 5%) and to establish a simple yet robust extraction methodology for the analysis of a wide selection of PFAS in whole blood.

Materials and Methods: Five extraction methodologies were evaluated for their extraction efficiency of 75 PFAS from DBS at 5 ng/mL (using horse blood). The methodologies were compared for their accuracy and precision statistically, to establish their extraction efficiency and viability at a whole blood analyte concentration. Two different protein precipitation methods (centrifugation (PPTc) and filtration via positive pressure manifold (PPTf)), an acid-base (liquid-liquid) extraction (LLE), a protease digestion (trypsin) with protein precipitation (centrifuged) (TPD), and weak anion exchange (OASIS WAX cartridge) solid-phase extraction (WAX) were investigated. The optimal extraction method was then applied to DBS samples collected daily from an individual donor of the research cohort (n = 7). Sample analysis was performed using an optimized LC-MS/MS method for 75 PFAS.

Results: Of the 75 PFAS investigated, PPTc recovered 60 and 65 within 70-130% and 50-150%, respectively, with a relative standard deviation less than 20% for 71 PFAS. Likewise, PPTf recovered 62 and 67, LLE 42 and 60, TPD 37 and 48, and WAX 47 and 59 PFAS, within 70-130% and 50-150% respectively (RSD < 20% (out of 75): PPTf – 73, LLE – 68, TPD – 44, WAX – 67). PPTf was then applied to the samples collected from the research cohort. 19 of the 67 PFAS were detected in the donor DBS, including C4-C11 and C13 perfluoroalkyl carboxylic acids (PFCAs) and C3-C8 perfluoroalkyl sulfonic acids (PFSAs). The dominant PFAS detected were both branched (Br) and linear (L) isomers of PFOS (Br-PFOS range: 1.4-1.8 ng/mL and L-PFOS range: 1.4-1.9 ng/mL), L-PFHxS range: 0.78-0.99 ng/mL and L-PFOA range: 1.2-1.5 ng/mL.

Discussion and Conclusion: The comparisons of the methods undertaken confidently identify PFAS extraction via PPTf as the most accurate and consistent extraction methodology from DBS. Furthermore, attesting to this observation is the benefit of method simplicity when dealing with small sample and processing volumes, eliminating opportunities for analyte loss and contamination. The capacity for confident extraction and analysis of 62 PFAS from DBS, coupled with non-invasive ‘self-sampling’ via a commercial Microsampler, are major advantages of the discussed extraction methodology when compared to existing blood PFAS analysis methods. A paradigm shift from target to non-target analysis is apparent for PFAS research, and both the sampling and extraction techniques discussed are readily suited for non-target applications.

References:

1. Introduction:
Analytical standards for per- and polyfluoroalkyl substances (PFAS), such as perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA), in water samples are published by the International Organization for Standardization (ISO 251011, ISO 216752), the Japanese Industrial Standard JIS K 0450-70-103, and the US Environmental Protection Agency (EPA) Method 5334 and Draft Method 1635. In particular, the ISO standard is widely used to reveal the global situation of PFAS contamination, including the PFAS marine pollution monitoring program of the United Nations Environment Programme (UNEP)6). However, there is no standard method for measuring PFAS in the atmosphere up to date. Volatile PFAS, such as 4:2 fluorotelomer alcohol (FTOH), are difficult to collect with commonly used high-volume air samplers, and as a result, many studies have evaluated non-volatile PFAS components collected on filters adsorbed onto particles such as house dust and PM2.5 as atmospheric PFAS. Therefore, accurately measuring volatile PFAS in the atmosphere was needed. This study presents a novel analytical method that uses a nano particle and gas sampler (NS20) and GC-Orbitrap-HRMS for the identification and quantification of volatile PFAS in ambient air samples.

2. Materials and Methods:
2.1 Sample collection and sample treatment
The sample collection and sample treatment method followed our previous publication7)8). The atmospheric samples were collected using NS20 (Sibata Scientific Technology Ltd. Scientific, Tokyo, Japan) with quartz fiber filters (QFFs; diameter 25 mm and 47 mm, Pall Corporation, NY, USA) for particle collection, and polyurethane foam (47 mm diameter, Sibata Scientific Technology Ltd. Scientific, Tokyo, Japan) and activated charcoal filters (GAIACTM; 47 mm diameter, Futamura Chemical Co., Ltd.) at 20 L/min flow rate. The ambient air samples of J1 (total sampling volume: 56.77 m3) and J2 (57.76 m3) were collected in Tsukuba City, Ibaraki Prefecture, and J3 (303.4 m3) was a composite of samples collected at the same location with five samples. Similarly, sample C1 (128.4 m3) was an atmospheric sample collected on Dongshan Island in Fujian Province, China, and C2 (564.6 m3) was a composite of samples collected in the same area with five samples.

For the development and validation of the instrumental measurement method for volatile PFAS, extract of GAIACTM was used. A mixture of 50 ng of each stable isotope-labeled standard was spiked onto GAIACTM before extraction, then extracted with 10 mL dichloromethane/ethyl acetate (1:1, v/v) with thoroughly soaked for one hour and repeated twice. The extract was concentrated to 1 mL under a gentle nitrogen stream. The recovery rates of the stable isotope-labeled reagents were 93-121% and 64-97% for samples collected in Japan and China, respectively.

2.2 Instrumental analysis
An instrumental method was developed using gas chromatography-high-resolution mass spectrometry (GC-Orbitrap-HRMS) (TRACE™ 1310 GC and Orbitrap™ Exploris™ GC Mass Spectrometer, Thermo Scientific, Bremen, Germany) for 36 organic halogen compounds, including volatile PFAS. GC separations were performed using DB-HeavyWAX (30 m × 0.25 mm i.d., 0.25 μm film thickness, Agilent Technologies, Santa Clara, USA), and an inert fused silica capillary tube (length 5 m, inner diameter 0.25 mm, Agilent Technologies, Santa Clara, USA) as the pre-column using the following temperature program: 60°C for 4 min, 5°C/min to 70°C, 3°C/min to 100°C, 8°C/min to 170°C, 20°C/min to 250°C then 40°C/min to 270°C for 8 min. The transfer line was maintained at 230°C. Electron ionization was performed at 30 eV or 70 eV with the source temperature set at 200°C. PTV injector was used in splitless mode with an injection volume of 2 μL. Full scan and SIM acquisitions were performed at the resolution of 30,000.

Data collection and analysis were performed using Chromeleon and Compound Discoverer 3.3 (Thermo Fisher Scientific). For evaluation of the comparability of Orbitrap spectra with existing EI-library spectra, NIST Mass spectral Library & Search Software was used.

Standard solutions were analyzed both GC-Orbitrap-HRMS and GC-triple quadrupole MS (GC-MS/MS) GCMS-TQ8050, Shimadzu, Kyoto, Japan for the instrumental limits of quantification (LOQ). Parameters of GC-MS/MS was described elsewhere7-9).

2.3 Matrix spike recovery test
A sample solution was prepared by adding of standard solution in methanol (50, 200, or 400 ng/mL) to 90 µL of atmospheric sample extraction solution to create a standard concentration of 5, 20, or 40 ng/mL in the sample. Control sample was also
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Prepared by adding 10 µL methanol to 90 µL of atmospheric sample extraction solution. SIM and full scan mode were performed using GC-Orbitrap-HRMS. The matrix spike recovery rate was calculated by comparison of control sample and standard adding samples.

3. Results and discussion:

3.1 The sensitivity and linearity of PFAS analysis using GC-Orbitrap-HRMS and triple quadrupole GC-MS

A total of twenty-two PFAS were compared I-LOQ to evaluate the sensitivity of both GC-Orbitrap-HRMS and GC-MS/MS using SIM acquisition. As a result, the I-LOQ of GC-Orbitrap-HRMS was found to be in the range of 0.025-0.25 ng/mL, which is comparable to or better than the I-LOQ of GC-MS/MS (0.05-5 ng/mL). Some compounds, including N-methyl perfluorooctane sulfonamide (N-MeFOSA), bromopentfluorobenzene (BPFB), and FTOH compounds other than 4:2 FTOH, showed clearly better I-LOQ using GC-Orbitrap-HRMS than GC-MS/MS.

The linearity of calibration curve was evaluated at a concentration range of 0.025 - 2.5 ng/mL for GC-Orbitrap-HRMS. The concentration range exhibiting a determination coefficient of 0.99 or higher was found to be narrowest within the 0.25-2.5 ng/mL range, and widest within the 0.025-2.5 ng/mL range.

3.2 Comparison of SIM mode and full scan mode of environmental atmospheric samples using GC-Orbitrap-HRMS

The measurement values may vary due to factors such as humidity, temperature, and matrix effects for the analysis of atmospheric samples. To verify the matrix effect on atmospheric samples analysis using SIM mode and full scan mode, a known concentration of standard was added to the atmospheric sample extract, and the resulting sample extract was analyzed to calculate the matrix recovery rate. It was observed that the matrix recovery rate was lower for atmospheric samples with a larger atmospheric sampling volume, and the matrix recovery rate in scan mode was lower compared to that in SIM mode for all atmospheric samples. The limited introduction of ions into the analyzer at one time to obtain high-precision mass spectra is a fundamental principle of Orbitrap. This is the reason for the reduced number of ions of the target substance in full scan mode as the analyzer is occupied by impurity ions. In contrast, SIM mode separates impurity ions from the sample using quadrupole mass separation before entering the analyzer. The use of SIM mode is effective in improving the analysis accuracy after identifying the ion and peak of unknown substances using full scan mode.

However, the matrix recovery rate was outside the range of 70-125% even in SIM measurement for some samples. In this study, atmospheric sample extract was measured without pretreatment such as cleanup. In order to conduct high-sensitivity measurements for targeted compounds, it is critical to use device conditions that minimize the impact of impurity ions during SIM measurement and to conduct purification through pretreatment and validation tests to verify the quantitative lower limit, as in conventional quantitative analysis.

3.3 Detection of volatile PFAS in ambient air from Japan and China

Volatile PFAS present in atmospheric samples collected with NS20 were measured in SIM mode using GC-Orbitrap-HRMS. Figure 1 shows the concentration of volatile PFAS present in the Japanese atmospheric samples J1, J2, and J3, and in the Chinese atmospheric samples C1 and C2. As a result, a total of 21 volatile PFAS were detected in the atmospheric samples of Japan and China at a concentration of 0.02 pg/m3, using the NS20 sampler and GC-Orbitrap-HRMS measurement method.

To the best of our knowledge, this is the first study to measure volatile PFAS such as 4:2 iodine, fluoroacetoloyl acrylates (FTAC), 6:2 FTAC, and 6:2 fluorotelomer methacrylates (FTMAC) in the ambient air samples using GC-Orbitrap-HRMS. Among the target compounds analyzed in this study, N-EtFOSA, 8:2 FTOH, 10:2 FTAC, 8:2 FTAC, 8:2 FTMAC, perfluorodecyl iodide (PFDeI), perfluorododecyl iodide (PFDoI), 8:2 fluorotelomer iodides (FTI), and 10:2 FTI are classified as first-class specified chemical substances under the Chemical Substances Control Law in Japan, which are classified as PFOA-related substances in the Stockholm Convention on Persistent Organic Pollutants (POPs).

The highest concentration of 8:2 FTOH was detected in China, followed by 6:2 FTOH. In Japan, not only was 6:2 FTOH present at a higher concentration than 8:2 FTOH, but high concentrations of 6:2 FTMAC, 6:2 FTI, 1,6-diodoperfluorohexane (PFHxDiI), BPFB, and other iodine and bromine-substituted compounds were also detected by J1 sample. This may reflect the situation of domestic industries that are developing alternatives to PFOS/PFOA. For example, FTOH, which is used as a material for surfactants and surface protectants, is manufactured from PFAI and FTI. FTAC and FTMAC known as FAAC and FAMAC in respectively are manufactured from FTOH and used in polymer manufacturing10). Additionally, BPFB is used as a pharmaceutical intermediate and is estimated to be released into the environment as a product, product residue, or decomposition product. In addition, 6:2 FTAC was detected only in the Japanese atmosphere, while dichlorobenzotrifluoride (DCTCB) was detected only in the Chinese atmosphere. These observations suggest that not only the production and use of FTOH, which are the major precursors of environmentally persistent PFOA and other PFAS, but also the production and use of bromine and iodine substituted PFAS should also be considered to understand the sources of PFAS in the atmosphere.
3.4 Suspect screening analysis of volatile PFAS and other compounds in ambient air samples using library search

The Orbitrap mass spectrometer is a Fourier transform MS known for its very high mass resolution and mass accuracy. It is capable of measuring with a mass resolution of 30,000 or higher and a mass accuracy of 3 ppm or less (1 ppm or less with the internal standard method). However, most of the spectral libraries commonly used in GC-MS analysis, such as the NIST EI spectral library, have low-resolution spectra like those of quadrupole MS. Therefore, searching a library based on high-resolution mass spectra may not provide sufficient precision mass spectra and may lead to incorrect search results. Software is available to build a library search with low-resolution spectra by converting the mass-to-charge ratio of the high-resolution spectrum to an integer value and then scoring the similarity of the spectra. Fragment spectra annotation can be performed against the high-resolution mass spectrum, enabling library search using a low-resolution spectral library. However, the most reliable analysis results can be obtained by constructing a high-resolution mass spectral library using the actual device used for the analysis.

A comprehensive analysis was performed on standard solution, ambient air sample extract with and without the addition of known standard compounds using Compound Discoverer 3.3 which is the software of a mass spectrometry data analysis. The analysis aimed to detect compounds in the samples that matched the standard substances via library search. Compounds that were detected with a spectral similarity score of 500 or higher are analyzed.

Generally, compounds with concentration of approximately 5 ng/mL were hit by library search in standard solution. However, only 8:2 FTOH, PFHxDoI, and 1,4-Diodoperfluorobutane (PFBuDiI) were identified by library search in the ambient air samples without the addition of standard substances, due to their low concentrations. PFHxDoI (chemical formula C6F12I2), which was identified by library search of the ambient air, had a spectral similarity score of 858 in Compound Discoverer 3.3, and the composition of 87% of fragment ions was estimated with high mass accuracy. On the other hand, some target compounds were detected in the ambient air sample extracts from J1, J2, and C1 when a known concentration of standards was added at 5 ng/mL, although most of the target compounds were detected at 20 ng/mL. Therefore, it is considered that a comprehensive analysis by library search is possible if the final solution concentration is 5 ng/mL, which corresponds to 80 pg/m3 or higher in actual atmospheric concentrations under the conditions used in this study. However, only a few compounds were detected in the ambient air sample extracts from J3 and C1 which composite samples with large amount of sample volume when a known concentration of standards was added at 20 ng/mL by library search. The amount of impurities increases along with the target compounds, in composited samples. It is believed that the ions of the impurities occupied the analyzer, resulting in a decrease in the amount of target substance ions introduced into the analyzer and subsequently reducing the sensitivity of the analysis for target compounds. To reduce the matrix effect, it is necessary to reduce impurities in the measurement sample by either cleaning up or diluting the sample. On the other hand, it is important to note that the cleanup process may also exclude unknown components, so the selection of pre-processing is an important parameter to consider.

4. Conclusions:
A new analytical method using GC-Orbitrap-HRMS was developed for the identification and quantification of volatile PFAS in ambient air samples. Thirty-six volatile PFAS and halogenated organic compounds in ambient air samples, which were collected using nano particle and gas sampler (NS20) in Japan and China in 2019 and 2020, were successfully analyzed by the GC-Orbitrap-HRMS method. This is the first study to measure volatile PFAS such as FTI, perfluoroalkyl diiodide (PFADiI), FTAC, FTMAC, BPFB and 1-bromo-3,5-bis(trifluoromethyl)benzene (BTFMBB) in the ambient air samples using GC-Orbitrap-HRMS. In addition, we examined matrix effects which prevent accurate quantification of volatile PFAS in ambient air samples, and then significant matrix effects were observed in MS scanning. The selection of analysis mode is one of the important parameters for GC-Orbitrap-HRMS. This technique will be enabled to understand the kinetic of PFAS in the ambient air.
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5. Acknowledgments:
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P-093 Passive Sampling Improves PFAS Monitoring in Wastewater

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Introduction: Passive sampling is a promising alternative to traditional approaches for sampling of PFAS in complex and variable waters such as wastewater and other effluents. The technique is affordable and simple to use both in a field setting and on the laboratory. The study hypothesized that passive sampling is beneficial for representative trace monitoring of PFAS by ensuring low detection limits while maintaining analyte integrity by minimizing the risk of biotransformation of labile PFAS such as Me/Et-FOSAA.

Materials and Methods: To assess the suitability of the passive sampler (binding efficiency and bias) commercially available passive samplers were exposed to controlled lab scale pilot tests. The water was spiked with PFAS compounds (certified reference standards) to an individual concentration of 10-40 ng/l and the samplers were incrementally incubated between 5 and 21 days. Samplers were subsequently placed in pure water for assessment of PFAS retention. For field tests, samplers were deployed in a municipal wastewater treatment plant (WWTP) at three key locations (influent, after biological treatment and effluent) during 7, 14 and 20 days. The sampler was composed of a diffusive hydrogel (agarose on a polyethersulphone membrane) and a weak anion exchange sorbent that could be isolated and extracted by sonication in methanol with 0.1 % ammonia for determination of PFAS. Up to 69 individual PFAS compounds were measured by LC-MSMS. The passive sampling approach was compared with traditional grab sampling and incremental composite sampling, analyzed by LC-MSMS after solid phase extraction.

Results: Lab scale tests concluded that the passive sampler was able to irreversibly retain the investigated PFAS compounds when placed in ultrapure water subsequent of incubation in contaminated water. Lab tests revealed that the sampling efficiency was comparable to direct grab sampling with proportional increase in passive sampler binding (sorbent concentration) of PFAS with deployment time. The adsorbed PFAS could be mathematically correlated and transformed to water phase PFAS concentrations.

A total of 19 PFAS compounds were detected in the field tests. None of the labile PFAS were detected. Though concentrations were low, the passive sampler indicated a negative bias in sampling of short PFAS.

Similar PFAS concentration trends were observed regardless of sampling method (fluctuations of PFAS concentrations were small during sampling). The total whole-water concentrations of PFAS were comparable from composite and grab sampling of wastewater influent but the passive sampler measured significantly less concentrations of PFAS. The results from sampling sites downstream the WWTP did not exhibit the same concentration difference between traditional sampling and passive sampling. More PFAS compounds could be detected by passive sampling than by composite and grab sampling. This effect was notable for longer chained PFAS. Additionally, the fluorotelomer 6:2 FTS was measured in significantly higher concentrations by passive sampling than by traditional sampling.

Discussion and Conclusion: The diffusive gradient passive samplers have been increasingly deployed for passive sampling of wastewater (1, 2). Despite a high loading of suspended particulate matter in the wastewater, the passive sampler was able to detect a wider range of PFAS, and notably longer chained PFAS, than detected through composite and grab sampling. Passive sampling is not representative for whole-water sampling in waters with high suspended particulate matter content as observed from sampling of influent of the WWTP. It is however a powerful tool for assessing dissolved, bioavailable water-phase, concentrations of PFAS in the presence of particulate matter. A drawback of the passive sampler is however that proper estimation of the dissolved concentration requires calibration by empirical measurements of diffusion coefficients and sampling rates (3).

Although 6:2 FTS is a potential biotransformation product from fluorotelomer thioether amido sulfonates, it is unclear why the concentrations of 6:2 measured higher by passive sampling than by traditional sampling. The observation needs to be studied further.

Acknowledgments:

References:
Per and Polyfluorinated Substances (PFAS): Occurrence and Exposure

P-094  Concentrations of PFAS in a community exposed to contaminated drinking water: the Pease Study

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Introduction: Aqueous film forming foam (AFFF) containing per- and polyfluoroalkyl substances (PFAS) was used in Portsmouth, New Hampshire, in suppressing fires and training exercises. This use resulted in the migration of PFAS through soils to groundwater and drinking water around the former Pease Air Force Base and later the Pease International Tradeport (“Pease”). An early biomonitoring study (2015–2019) detected elevated perfluorohexane sulfonate (PFHxS), perfluorooctane sulfonate (PFOS), perfluorooctanoate (PFOA), and perfluorononanoate (PFNA) serum concentrations in persons who worked and drank water at Pease before the contamination was remediated in 2014 (Daly et al., 2018).

Materials and Methods: In this study we examined serum PFAS concentrations in 776 adults to assess the PFAS exposure from contaminated drinking water in the area. Persons who worked at Pease were eligible including those who participated in the earlier biomonitoring effort. Data were collected in the years 2019–2021. Nine PFAS concentrations (PFHxS, linear and branched PFOS, linear and branched PFOA, PFNA, perfluorodecanoate (PFDA), perfluoroundecanoate (PFUnDA), and 2-(N-methyl-perfluorooctane sulfonamido) acetate (MeFOSAA)) were measured at the National Center for Environmental Health using online solid phase extraction-liquid chromatography-isotope dilution-tandem mass spectrometry (Kato et al., 2018).

Results: PFAS serum geometric mean concentrations in our cohort will be compared to geometric means from the 2017-2018 National Health and Nutritional Examination Survey (NHANES) participants. The serum concentrations from this cohort will be compared to other studies which have examined differences in serum PFAS concentration levels by sex and age.

We will explore whether increased tap water consumption at Pease Tradeport during the years of PFAS contamination correlates to higher PFAS serum concentrations. Questionnaire responses will be used in to estimate the number of cups of tap water consumed on the Pease Tradeport between 1992 and 2014. Pease study cohort PFAS geometric mean concentrations will also be compared to past measurements from the NH biomonitoring program for the participants who were enrolled in both studies.

Discussion and Conclusion: In the prior study, PFOS, PFOA, PFNA, and PFHxS serum concentrations were elevated in this cohort. Those are the molecules typically found in AFFF. We will investigate whether those analytes are still elevated. We will also investigate whether PFAS serum geometric mean concentrations are higher among Pease participants than NHANES 2017–2018 participants. For the participants with two PFAS measurements, we will evaluate if these measurements follow the trends observed in NHANES which show decreases over time.

Acknowledgements: We thank the study participants taking part in the study.
The findings and conclusions in this report are those of the author(s) and do not necessarily represent the views of the Centers for Disease Control and Prevention or the Agency for Toxic Substances and Disease Registry.

References:
Per- and polyfluoroalkyl substances (PFAS) are a class of thousands of substances that have been produced since the 1940s and used in a wide range of consumer and industrial products. PFAS are of concern because of their high persistence and their impacts on human and environmental. The occurrence of PFAS is related to urban and industrial discharges, and due to their high water solubility, surface or groundwater is a significant medium for long-range transport. The State of São Paulo is the most industrialized region in the country, with a population of more than 40 million people, and as an impact of the State’s heavy industrialization, disposal of undesirable toxic wastes and discharge of sewage and industrial effluent constitutes major sources of surface water pollution. In 2009, PFOS have been included in annex B as a persistent organic pollutant to the Stockholm Convention, PFOA and PFHxS have been included in annex A in 2019 and 2022, respectively. There is no regulation related to PFAS in Brazil and the National Implementation of the Stockholm Convention in Brazil indicate that the sulphuramide, a probable precursor of PFAS, is still allowed to use as insect baits for leafcutter ants from Atta spp. and Acromyrmex spp. There is a lack of information about the use and environmental levels of PFAS in Brazil and this study was performed in order to fill the data gap on the occurrence of PFAS in the São Paulo State water bodies.

A total of 41 surface water (16 sites) and 23 groundwater (16 sites) samples were collected at multiple locations from rivers, reservoirs and ground water wells located at different Watershed Management Units (WMU) within the São Paulo State to determine the presence of PFOS, PFOA and PFHxS, during the period of 2021 and 2022. Water samples were spiked with isotopically labeled compound standards and were extracted using solid phase extraction with Oasis HLB 6cc (200 mg) cartridges. PFAS were eluted with methanol and concentrated using a gentle stream of nitrogen. Final extracts were filtered directly to an insert using Acrodisc syringe filters (13 mm, 0.2 µm GHP). Isotopically labeled internal standards were added before injection and analyzed on an ultrahigh performance liquid chromatograph coupled to a quadrupole time of flight mass spectrometry (UPLC-QToF, G2-XS, Waters). The UPLC was fitted with an Acquity UPLC BEH C18 1.7 µm column (2.1 x 100 mm). The identification and quantification were performed using isotopic dilution method. Recoveries of isotopically labeled compound standard added before extraction were within the acceptance limits described in U.S.EPA 1633. Blanks and spiked native PFAS standard control were conducted with each batch samples and analyzed under the same conditions than samples. The laboratory participates in international interlaboratory studies in order to certify the quality of the results obtained.

The PFOS concentrations in surface water samples ranged from <LOQ – 11.9 ng/L, PFOA from <LOQ–10.0 ng/L and PFHxS from <LOQ – 1.84 ng/L; PFOS concentration in ground water samples ranged from <LOQ – 2.69, PFAO from <LOQ – 1.99 and PFHxS from <LOQ – 1.31 ng/L. There is no regulations for PFAS in Brazil but the results found in this study are lower than US EPA draft Water Quality criteria for PFOS and PFOA for the protection of aquatic life and for the ground water quality criteria. The PFAS monitoring in surface and ground water still continue to have a better assessment of the fate and distributions of PFAS in São Paulo State water bodies.

We gratefully acknowledge the support and infrastructure provided by CETESB and all the people who worked on this study. The authors also acknowledge the funding from State Water Resources Fund (FEHIDRO), grant number 062/2015 - COHRI-156.

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Per and Polyfluorinated Substances (PFAS): Occurrence and Exposure

P-096 Survey of neutral PFASs in air using FM4 air sampler in five regions of Japan

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1. Introduction:
The estimated sources of perfluoroalkyl compounds (PFASs) include the manufacturers of these fluorosurfactants, fluorinated resin manufacturing plants that use these products (especially perfluorooctanoic acid: PFOA), plants that perform surface treatment, especially in the fiber and textile industry, semiconductor-related and other electronic material-related companies, metal plating and etching-related plants, paper and paper industry, rubber and plastic-related plants [1]. Depending on the synthesis process and raw materials, commercially produced perfluoroalkyl sulfonic acids (PFSAs) and perfluoroalkyl carboxylic acids (PFCAs) products are estimated to have at least 4 to 13 carbon homologues [2]. These compounds, like perfluorooctane sulfonic acids (PFOS) and PFOA, are also detected in a variety of environments. Organofluorine compounds that are considered to be precursors of PFOS/PFOA, such as sulfonamides (FOSAs, FOSEs, etc.) and telomer alcohols (FTOHs), have also been found to be widely present in the environment.

The first wide-area survey on atmospheric PFASs in Japan was conducted by the Ministry of the Environment in 2004 as "The Environmental Survey for Exposure Study" [3]. The survey was conducted for PFOS and PFOA at 20 sites (57 samples) in Japan. Subsequently, surveys have been conducted continuously in "The Environmental Survey and Monitoring of Chemicals" conducted by the Ministry of the Environment in Japan since FY2010 [4]. However, the current survey is limited to PFOS/PFOA and does not include other PFASs, which are related compounds.

Some compounds of PFASs are being used as alternatives to PFOS and PFOA, which are being addressed worldwide through reduction plans associated with their designation as POPs, but their actual status is not well understood. In this study, an actual environmental survey was conducted in five regions in Japan (Fig. 1) on PFASs in the air, for which very little information is available, using the FM4 air sampler [5], which can simultaneously collect ionic and neutral PFASs. In this report, we describe the results of neutral PFASs among the PFASs.

2. Materials and Methods:
The FM4 air sampler used for the PFASs study in air can classify particles into three sizes at a flow rate of 20 L/min: >10 µm (first stage), 2.5 to 10 µm (second stage), and 1.0 to 2.5 µm (third stage), and uses quartz fiber filter (QFF), polyurethane foam (PUF) and activated carbon filter disk (GAIAC®) are used as collection materials (Fig. 2), and a low volume air sampler (LV) is used to collect ambient air [5]. In this study, we have investigated more than 30 substance groups based on ISO 21675, of which 18 were neutral PFASs (Table 1). The survey was conducted three times at five sites in Japan, each from April to July. Extraction of QFF, PUF, and activated carbon filter disks were all performed with 50% dichloromethane/ethyl acetate.

The eluate was concentrated under nitrogen gas flow to a final volume for instrumental analysis. Quantitation and identification of neutral PFASs were determined by GC-MS/MS (Agilent Technologies: 7010 TripleQuad GC/MS) (Table 2). Quantification was performed by the internal standard method for substances for which labelled compounds were available (4:2 FTOH, 6:2 FTOH, 8:2 FTOH, 10:2 FTOH, N-MeFOSE, and N-EtFOSE) and by the absolute calibration curve method for substances for which labelled compounds were not available (other than the above).
3. Results and Discussion:
The results are shown in Figure 3. The concentration range of neutral PFASs in the air was 60-1000 pg/m³, with an average value of 470 pg/m³. The concentration ranges for each group were 47 to 1000 pg/m³ for FTOHs, N.D. to 82 pg/m³ for FTIs, N.D. to 91 pg/m³ for FDIAs, and FOSEs, FOSAs and PFDoIs were not detected. Of the 18 substances measured, 10 were detected. Four of the 10 substances detected were FTOHs such as 6:2FTOH and 8:2FTOH. Among them, 6:2FTOH was detected in all samples and showed higher concentration levels than the other FTOHs. 8:2FTOH is one of the volatile precursors of PFOA, a compound covered by the Stockholm Convention on Persistent Organic Pollutants (POPs Convention). 8:2FTOH was also detected in all samples and was second in priority after 6:2FTOH in many samples. Wu et al. [6] conducted a study on PFASs in ambient air in Tsukuba city, Japan, and reported that FTOHs were dominantly detected among 19 neutral PFASs, with 6:2FTOH concentrations accounting for 40% and 8:2FTOH for 32% of total neutral PFASs concentrations. Because 4:2FTOH is highly volatile, it is estimated that most of it exists as a gas in the atmosphere. Therefore, it was difficult to collect 4:2FTOH with glass fiber filter or quartz fiber filter, but by using the FM4 air sampler and activated carbon filter disks (GAIAC®), we were able to detect it at four of the five sites surveyed.

With regard to seasonal changes in the concentration of neutral PFASs in the atmosphere, the results [7] of the survey in February 2022 (cold season) and the results of this survey (warm season) were compared. The sampling sites were the same sites in Osaka regions in Japan for both the 2022 and this survey, and the sampling methods were also the same. Four out of 18 substances were detected during the cold season, while eight substances were detected during the warm season. Comparing the concentration levels of 6:2FTOH and 8:2FTOH, which were detected in both cold and warm seasons, 6:2FTOH ranged from 51 to 180 pg /m³ (average 120 pg /m³) in the cold season versus 190 to 430 pg /m³ (330 pg /m³) in the warm season, and 8:2FTOH ranged from N.D. ~ 40 pg /m³ (13 pg /m³) in the cold season versus 57-110 pg /m³ (75 pg /m³) in the warm season. These results suggest that most of the neutral...
Per and Polyfluorinated Substances (PFAS): Occurrence and Exposure

P-096  Survey of neutral PFASs in air using FM4 air sampler in five regions of Japan

PFASs are present in the atmosphere during warmer periods.

Furthermore, with regard to the variation in concentrations between five regions, a difference of approximately 10-fold was observed between the high and low average total concentrations of neutral PFASs. However, common trends were also observed, such as 6:2FTOH and 8:2FTOH being predominantly detected in all regions and FDIAs also being detected in all regions.

Taniyasu et al. [8] also detected 21 volatile PFASs in air samples from Japan and China using gas chromatograph high-resolution mass spectrometer (Thermo Fisher Scientific: GC-Orbitrap-HRMS). The results of this study, together with those of other studies, indicate that a wide variety of volatile neutral PFASs are present in the air in Japan.

4. Conclusions:
Most of neutral PFASs under investigation were collected on activated carbon filter disks (GAIAC®), although some compounds were collected on PUF. The method used in this study with activated carbon filter disks proved to be effective for measuring highly volatile PFASs in the air. In the future, we plan to clarify the actual status of PFASs in the air by expanding the survey sites and conducting the survey during the warmer season.

Focusing on neutral PFASs, which are suspected to eventually transform into POPs such as PFOS and PFOA, will help manage the risk of these compounds in the long term. It is important to continue to confirm their disappearance.

5. Acknowledgments:
This research was supported by the Environment Research and Technology Development Fund (JPMEERF20211G02) of the Environmental Restoration and Conservation Agency provided by Ministry of the Environment of Japan.

6. References:
1. National institute for environmental studies, Japan, Report of special research from the National institute for environmental studies, Japan "Study on the establishment of scientific and technical foundation for assessment of sources, development of destruction method and elucidation of pollution status of POPs-like compounds, especially organofluorine chemicals" (2006)
Per and Polyfluorinated Substances (PFAS): Occurrence and Exposure

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Introduction:
In the last decades, per- and polyfluoroalkyl substances (PFAS) have entered the environment and have been found in soil, groundwater and sediments where they pose a threat to human health and ecosystems. These substances need to be managed and remediated to prevent risks and further spread. For this reason, OVAM (The Public Waste Agency in the region of Flanders, Belgium) has commissioned VITO to propose soil remediation values (SRV) for Perfluorooctane sulfonic acid (PFOS) and Perfluorooctanoic acid (PFOA).

Materials and Methods:
A generic approach following guidelines is used to derive the human health-based soil remediation values. The calculations of the SRV’s were carried out with an adapted version of S-Risk© 1.3, a model for human exposure and health risk assessment at contaminated sites which calculates the fate and distribution of chemical pollutants in soil. To run the model, data were collected in the literature regarding the behavior of PFOS and PFOA in soil and physicochemical properties, exposure via air, transfer to plants and animals and legal limits. Information concerning the toxicological reference value used for the calculation of the SRV’s and the exposure via food were taken from EFSA (EFSA, 2020). The threshold is a group tolerable weekly intake (TWI) of 4.4 ng/kg bw per week for PFOS, PFOA, PFNA (Perfluorononanoic acid) and PFHxS (Perfluorohexane sulfonic acid). Since EFSA does not indicate how these 4 PFAS should be weighted relative to each other, no ‘distribution’ of the TWI across the 4 PFAS compounds is taken into account, and SRV’s are calculated using 4.4 ng/kg bw per week.

The default land use types for which values were calculated are agriculture, residential, recreation and industry. The S-Risk© model calculates soil concentrations for each land use type that corresponds to an exposure which results in a risk index equal to 1. Calculations were performed for 2 age groups: young children (1 to 6 years) and adolescents/adults from 15 years onward.

Results:
Table 1 shows the human health based SRV’s calculated for 4 different land use types. Calculations were performed for children and for adolescents/adults as the most sensitive population.

<table>
<thead>
<tr>
<th></th>
<th>Agriculture</th>
<th>Residential</th>
<th>Recreation</th>
<th>Industry</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFOS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Children</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Adolescents / adults</td>
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<td>268</td>
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<td>PFOA</td>
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<td></td>
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<tr>
<td>Children</td>
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<td>4.0</td>
<td>245</td>
<td>-</td>
</tr>
<tr>
<td>Adolescents / adults</td>
<td>0.6</td>
<td>7.9</td>
<td>632</td>
<td>303</td>
</tr>
</tbody>
</table>

Table 1: Human health-base soil remediation values for PFOS and PFOA (µg/kg dm), calculated with S-Risk© for Flanders (Belgium).

Discussion and Conclusion:
Since the background dietary exposure for the 4 EFSA PFAS together already exceed the TWI, it can be concluded that each SRV (i.e. each additional exposure dose) entails a certain health risk. No SRV’s can be calculated for children for PFOS as the background dietary exposure already exceeds the EFSA TWI. However, SRV’s can be calculated for PFOA for children, since the background dietary exposure is smaller than the TWI. For adults, the background dietary exposure for PFOS and PFOA is always lower than the TWI and, consequently, SRV’s can be derived. When the calculated values for the agricultural land use type are compared with the target values for Flanders (= background values in soil), it appears that the calculated values for both PFOS (1.5 µg/kg dm) and PFOA (1.0 µg/kg dm) are below these target values. Based on the above calculations, additional information on ecotoxicology and practical feasibility, OVAM established a standards framework for PFOS and PFOA in soil in Flanders.

References:
**Introduction:** Perfluoroalkyl and polyfluoroalkyl substances (PFAS) are a diverse class of chemicals that have been widely used in industrial and commercial applications including in cookware, carpets, textiles and leathers, or used as lubricants, surfactants and firefighting foams since 1950.1,2 PFAS have aroused concerns owing to their persistence, bioaccumulation and toxicity (PBT properties).3,4 Due to the complicated interference in the soil and the low concentration of PFAS in soil (about ppt to ppb), the development of appropriate pretreatment methods to remove the matrix and concentrate the analyte is critical. In this study, we established an automated pretreatment process of on-line solid phase extraction (SPE) coupled with liquid chromatography high-resolution quadrupole Fourier transform electric field orbital mass spectrometry (LC/MSMS). A small amount of soil extract (1 mL) was added to reagent water, and then introduced into the instrument with an autosampler. The advantages of automated analysis can simplify the sample pretreatment process and reduce the chance to expose to contaminants such as Teflon or latent perfluoroalkyl substances during the extraction and concentration process. The method detection limit, the average recoveries of quality control (QC) samples, the average recoveries of spiked samples, and the relative difference percentage (RPD%) of the duplicate sample were evaluated in this study.

**Materials and Methods:** 2 g dry soil was taken to a 15 mL PP centrifuge tube and internal standard was added. Ten mL 50% methanol water solution with 0.5% ammonia as solvent were added for extraction by horizontal shaker (400 rpm for at least 4 hours). After centrifugation, draw 1 mL of centrifuged extract into a 10 mL glass sample bottle and add 150 µL of 10% formic acid methanol water solution with 0.5% ammonia as solvent were added for extraction by horizontal shaker (400 rpm for at least 4 hours). After centrifugation, draw 1 mL of centrifuged extract into a 10 mL glass sample bottle and add 150 µL of 10% formic acid aqueous solution and 9 mL reagent water for further on-line SPE LC/MSMS analysis. Introduce 5000 µL of sample extract with automatic injection system. One pump was used to bring the sample into the solid phase extraction column. The other pump was used to elute the analytes adsorbed on the solid phase extraction column and then the analytes flow into analytical column for separation and finally into the LC/MSMS. The entire time for extraction, separation, and analysis takes only 14.3 minutes. 29 PFAS includes 7 subclasses: perfluoroalkyl carboxylic acid (PFCAs), perfluoroalkyl sulfonic acids (PFSAs), fluorotelomer sulfonates (FTSAs), perfluoroalkane sulfonamide (FASA), N-alkyl perfluoroalkane sulfonamides (N-alkyl FASAs), N-Alkyl perfluoroalkane sulfonamide ethanols (N-alkyl FASEs), and N-alkyl perfluoroalkane sulfonamido acetic acids (N-alkyl FASAAs).

**Results:** An analytical method for detection of 29 per- and polyfluoroalkyl substances (PFAS) in soil by on-line SPE LC/QE was established. Twenty-nine PFAS including 11 PFCAs, 8 PFSAs, 3 FTSAs, 1 FASA, 2 N-alkyl FASAs, 2 N-alkyl FASEs, and 2 N-alkyl FASAAs were able to analyze in one sample run. The method detection limits for 29 PFAS ranged from 0.061 µg/kg dw to 0.098 µg/kg dw. The average recoveries of quality control (QC) samples were from 68% to 118%, the average recoveries of spiked samples were from 60% to 135%, and the relative difference percentage (RPD%) of the duplicate sample is less than 9%.

**Discussion and Conclusion:** The analytical time is only 14.3 minutes for complete on-line SPE and detection of LC/MSMS and the QA/QC data provides the robustness of this method. Twenty-nine PFAS including acidic and neutral PFAS were capable to analyze simultaneously which means that a sensitive, versatile, and time-saving methodology for analysis of PFAS in soil were successfully developed.

**Acknowledgments:** We would like to thank for the financial support provided from Environmental Protection Administration, Executive Yuan of R.O.C. (Taiwan), Soil and Groundwater Pollution Remediation Fund Management Board.

**References:**

Per and Polyfluorinated Substances (PFAS): Occurrence and Exposure

P-099 Revamping OTM-45 to measure PFAS-emissions – Lessons learned

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Introduction: Per- and polyfluoralkyl substances or PFAS or synthetic organofluorine chemicals. The chemical family contains over 6000 PFAS are widely used as they have unique desirable properties. Scientists and governments around the world have recognized the harmful effects of PFAS on human health and the environment. They are stable under intense heat, many of them are also surfactants and are used as water and grease repellents. Global occurrence and distribution of PFAS has been the subject of research for many decades, however it is not yet well understood. PFAS are not yet routinely monitored in flue gasses. VITO was asked by the Flemish government to develop a compendium reference method for the quantification of PFAS in flue gasses. As the Flemish reference laboratory for environmental analyses and measurements, VITO represents the link between the Flemish government and the environmental laboratories in Belgium.

Materials and Methods:
From the 3M PFAS crisis in 2021, VITO started gaining practical experience with the OTM-45 methodology1, published by the US EPA, in combination with the existing compendium analysis method for the quantification of PFAS via LC-MS/MS (WAC/IV/A/025) in water samples. A sampling train based upon OTM-45 was assembled, (partially) optimized and validated and applied in real large-scale industrial settings. Seeing the need for urgency of deployment full optimization and validation was not possible at the start of the sampling campaigns. The sampling was initiated at various incinerator sites and the method was optimized along the way.

Results: The sampling set-up consisted of a sampling probe connected to the stack, a filter, an XAD2 cartridge followed by a condenser, 3 impinger recipients containing water and finally an additional XAD2-cartridge. Mass-labelled PFBA, PFOA and PFOS were spiked on the first XAD2 cartridge to serve as sampling recovery standard. There recovery standards were used to ensure monitoring of the sampling efficiency.

VITO has conducted emission measurements of 50 different compounds on different stacks and industries in Flanders and gained initial experience on PFAS concentration levels, composition and potential impacts of gas scrubbing. In collaboration with other environmental laboratories, VITO adapted and implemented the OTM-45 methodology for validation of the proposed compendium method in order to arrive at a fit-for-purpose, scientifically sound and useful methodology for the quantification of PFAS (> C4, boiling point > 100 °C) in ducted gas streams. For reasons of confidentiality sampling locations and stacks cannot be identified. The only information that can be shared is that samples were taken at waste incinerators. Typical PFAS patterns were recorded at these incinerator sites, the main compounds being the PFCAs ranging from C4 to C18 (higher values starting from PFNA) and in smaller amount HFPO-DA. Almost no or very small levels of PFSAs, FTSs and PFOSA's were detected. Measured compounds were present at concentrations up to more than 1,000 ng/m³. The recovery standards were all found to be higher than 50 % upon analysis so the sampling efficiency was considered adequate.

Discussion and conclusions: The wide scope of PFAS with varying physico-chemical properties has brought to light that straightforward implementation of the existing OTM-45 method for PFAS will not always result in fit-for-purpose data of sufficient quality. Depending on the PFAS load of the chimney, the nature of the flue gasses and the chimney technology that is used, the sampling efficiency can be highly affected. Further, sampler and adsorbent preparation play a crucial role in the final data quality. In this work we present the caveats encountered during development, the proposed solutions and some insights in the first emission PFAS emission measurements in Flanders.

Introduction: Perfluoroalkyl and Polyfluoroalkyl Substances (PFAS) are a large group of synthetic compounds containing chains of linked carbon and fluorine atoms. They are used for a variety of applications, including, but not limited to, non-stick cookware, food packaging, cosmetics, water-proof clothing and flame-retardants. Due to their ubiquitous use, environmental exposure for humans to PFAS can come from numerous sources, including house dust, food and drinking water. Of these, drinking water is one of the most important pathways for human exposure. Exposure to PFAS has been linked with a variety of health effects, including altered immune and thyroid function, liver disease, lipid and insulin dysregulation, kidney disease, adverse reproductive and developmental outcomes, and cancer. In December 2020, the European Parliament and Council of the European Union released a new directive that sets the limit of PFAS in drinking water to 0.5 µg/L for all PFAS compounds identified, and 0.1 µg/L for a subset of PFAS compounds that are particularly concerning for humans. Due to rapidly evolving regulatory initiatives across various regions and countries, the number of PFAS compounds that will require monitoring is expected to increase. This makes it challenging for laboratories to stay current with their PFAS analysis and requires frequent method protocol updates.

Thermo Fisher Scientific has developed a LC-MS/MS method for the quantitation and confirmation of 54 PFAS compounds, with Limit of Quantitation (LoQ) at 0.2 ng/L by direct injection in both drinking and non-drinking water matrices. The method can meet the regulatory requirements, both in terms of PFAS monitored and LoQs, for all EU regulations.

Materials and Methods: A direct aqueous injection method was designed for drinking, surface, and ground water samples using Thermo Scientific™ Vanquish™ Core UHPLC system coupled to a Thermo Scientific™ TSQ Altis Plus™ triple quadrupole mass spectrometer. After the addition of surrogate standards and a simple dilution with methanol, 500 µL of the sample was injected directly onto the Acclaim RSLC Polar Advantage column.

Results: In general, large-volume injection methods are less common for PFASs compared to offline extraction methods, therefore we report the accuracy, repeatability, and recovery of several months of continuous water sample analysis to demonstrate the robustness and accuracy. Method detection limits were determined using drinking water samples and ranged from 0.05 to 1000 ng/L for 54 PFAS. In drinking water, all calibration points exhibited good accuracy within +/- 20% of the expected values for all points, and R2 coefficients >0.990. The inter batch precision and recovery for these analytes in drinking water and surface water were within the acceptable limits of 20% RSD at respectively 5 ng/L and 1 ng/L. This confirmed that the method applicability for a routine and more comprehensive analysis to allow an expanded scope of PFAS testing in these different water matrices.

Discussion and Conclusion: An analysis workflow using Thermo Scientific Vanquish Core UHPLC system coupled to a Thermo Scientific TSQ Altis Plus triple quadrupole mass spectrometer was developed. This makes the analysis of more than 50 PFAS compounds by direct injection in both drinking and non-drinking water possible within a range of 0.05 to 1000 ng/L. Direct injection has the advantages of minimal sample preparation and smaller risk to introduce PFAS contamination. The high acquisition rate of the TSQ Altis Plus (upto 600 SRMs per second) gives additional analytical capacity to increase the number of PFAS monitored. The method fulfils the regulatory requirements, both in terms of number of PFAS monitored and LoQs, for all appropriate EU regulations.

References:
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Per- and Polyfluorinated Substances (PFAS): Toxicity

P-101  The effect of PFHxS on the proliferation of human hepatocellular carcinoma cells

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Introduction: Perfluoroalkyl substances (PFAS) are a group of synthetic chemicals that are widely used in industry and consumer products. Recently, perfluorohexane sulfonic acid (PFHxS), a replacement PFAS, was added among persistent organic pollutants (POPs) and has been regulated worldwide as a new threat to human health. Animal studies have suggested that PFHxS induced liver steatosis and hepatocellular hypertrophy. However, human studies do not provide an evidence of the association of PFHxS with liver toxicity due to the limited number of studies. The aim of the present study is primarily to contribute to human health risk assessments by examining the effects of PFHxS on human hepatocellular carcinoma (HCC) cells, Hep3B and SK-Hep1.

Materials and Methods: Cells were treated with different concentrations of PFHxS and the proliferation of cells were evaluated by measuring cell viability, colony formation and the level of cell cycle signaling molecules.

Results: Cell viability was significantly increased at ≤ 200 µM PFHxS, while PFHxS decreased cell viability compared to control at > 400 µM. Similarly, PFHxS significantly increased colony formation at ≤ 300 µM, while PFHxS at 500 µM significantly decreased colony formation of HCC cells. Consistent with the effect of PFHxS on HCC proliferation, PFHxS significantly increased proliferating cell nuclear antigen (PCNA), a marker of proliferation, and cell cycle molecules driving quiescent G0 phase to the growth phase including cyclin-dependent kinase (CDK)2, CDK4, cyclin E, and cyclin D1.

Discussion and Conclusion: The effect of PFHxS on HCC is biphasic; PFHxS increases survival and proliferation of HCC cells at lower concentrations (below 200 µM) and induces cytotoxicity at higher concentration (above 400 µM). Our finding suggests PFHxS may exacerbate HCC progression at certain range of concentrations and may provide a new possible environmental factor to increase HCC risk.

Acknowledgments: This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (No. 2022R1I1A3071854).

References:
Per- and Polyfluorinated Substances (PFAS): Toxicity

P-102  Comparative Metabolomics of Short-term Maternal Gestational Exposure of Sprague-Dawley Rats to Emerging Perfluoroalkyl Ether Carboxylic Acids

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Introduction: Emerging perfluoroalkyl ether carboxylic acids (PFECA) such as hexafluoropropylene oxide dimer acid (HFPO-DA), perfluoro-3,5,7-butaoxadecanoic acid (PF04DA), and perfluoro-3,5,7,9,11-pentaoxadecanoic acid (PF05DoA) and others have been detected in the environment in recent years as nations work to restrict PFAS and corporations find replacements for legacy per- and polyfluoroalkyl substances (PFAS) of concern. U. S. Environmental Protection Agency is conducting toxicity assessments to better understand potential risks posed by the growing number of replacement PFAS as well as legacy PFAS that may threaten human health, especially for vulnerable populations. We previously reported developmental toxicity of Nafion byproduct (NBP2) compared to HFPO-DA and perfluorooctane sulfonic acid (PFOS) (Conley et al., 2021) short-term gestational exposure of rats to PF04DA and PF05DoA.

Materials and Methods: Pregnant Sprague-Dawley rats were dosed with either PF04DA (0 – 62.5 mg/kg/day) or PF05DoA (0 – 30 mg/kg/day) by oral gavage form gestational days (GD) 18-22. Maternal serum was collected on GD22. Serum samples were prepared and analyzed using the Biocrates MxP Quant 500 kit (Biocrates Life Sciences AG, Innsbruck, Austria). Sample extracts were analyzed by flow injection analysis (FIA) and liquid chromatography/tandem mass spectrometry (LC/MS/MS) on a Sciex (Framingham, MA, USA) 6500+QTRAP linear ion trap mass spectrometer system. Raw data were processed using Sciex Analyst version 1.7.2 and MultiQuant version 3.0.2. Quality control validation was performed using Biocrates MetIDQ (Oxygen version) software. Data were further processed using Sciex MarkerView version 1.3.1 and MetaboAnalyst version 5.0 (Pang et al., 2021).

Results: Gestational exposure to the structurally similar PFECA, PFO4DA and PFO5DoA, yields very different serum metabolomics results. Statistical analysis of all dose levels using one-way ANOVA with a p value cut-off of 0.05 (False Discovery Rate) indicates no significant features for PFO4DA. For PFO5DoA, 181 features were significant, including the anti-oxidant indole-3-propionic acid (3-IPA) (p = 3.09e-7), amino acids tryptophan (p = 0.000318) and 1-methylhistidine (p = 0.000318), bile acid taurochenodesoxycholic acid (TCDCA) (p = 0.00251), and multiple triacylglycerides and cholesterol esters. Significance testing of the highest dose for each PFECA versus the control demonstrated no significant differences for PFO4DA and 130 changed features for PFO5DoA. Fold change analysis revealed greater than 2-fold increase in cholic acid compared to controls for PF04DoA, with 25 metabolites, including tryptophan, 3-IPA, taurocholic acid (TCA), and TCDCA exhibiting greater than 2-fold increases. Greater than 2-fold concentration increases were observed for 43 metabolites, including cystine, cholic acid, and glycocholic acid, and multiple triglycerides for PF05DoA high dose exposed serum. PF05DoA exposure was related to concentration decreases of more than 2-fold for 81 metabolites, including 3-IPA, 3-IAA, tryptophan, and several cholesterol esters and phosphatidylcholines.

Discussion and Conclusion: The metabolic changes we observed after gestational exposure suggest very different biological interactions occur for PF04DA and PF05DoA. Significant concentration changes were observed for many metabolites after PF05DoA exposure, clearly differentiating exposed serum from controls. The data suggest dose-related impacts of PF05DoA exposure on liver function, lipid homeostasis, and oxidative stress response, and lesser disruption to metabolism after PF04DA exposure. Similarly, we observed greater metabolic disturbance related to gestational exposure to HFPO-DA and PFOS than NBP2.

Disclaimer: The views expressed in this presentation are those of the authors and do not necessarily reflect the views or policies of the U.S. EPA. Mention of trade names is not a recommendation or endorsement.

References:
**POPs and Microplastics**

**P-103  Presence of Persistent Organic Pollutants (POPs) in plastic litter from surface water in Callao bay, Peru.**

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**Introduction:** Plastic pollution is a significant global environmental issue that poses a severe threat to marine organisms. The adsorption of persistent organic compounds (POPs) by microplastics can result in chronic effects, mutations, and cancer risks in marine organisms, which can further impact the food web and entire ecosystems (Hirai et al., 2011; Kwon et al., 2017). Callao Bay in Peru, which houses industrial companies and is the country’s primary port, experiences high levels of pollution despite the presence of artisanal fishing and tourism. The bay’s sediment and various species contain high concentrations of DDTs and PCBs, especially near river mouths (Cabello & Sanchez, 2006; Martinez & Jacinto, 1997). However, there is still limited knowledge about the relationship between plastic litter and POPs in Callao Bay, as well as the extent of plastic litter in surface seawater, making further research necessary. The purpose of this study is to quantify the presence, distribution, and characteristics of microplastics in the surface waters of Callao Bay.

**Materials and Methods:** A cost-effective, homemade Low-Tech Aquatic Debris Instrument (LADI) trawl was used for sampling, costing approximately USD 150 to manufacture. The LADI trawl was modified to use anti-aphid nets with a size of 300-micron pore size (#50 mesh). Samples were collected from a 2 km stretch of surface water, parallel to the shoreline, at a speed of 1.5 to 2 knots. The measurements were made in a single day in mid-February 2023. At the beginning of each sampling, pH, conductivity, and dissolved oxygen measurements were taken. The six collected samples were stored, separated, filtered, dried, and analyzed by visual inspection and weighing of the particles, which presented various shapes and colors. One set of samples was designated for characterization, while the other was allocated for instrumental analysis, conducted at RECETOX labs.

**Results:** The findings highlighted the mouth of the Chillon River as the area with the highest incidence of particles, with 4.4 million particles per square kilometer or 82 particles per cubic meter. Evidence of what were found is shown in Figure 1. Their investigation yielded some concerning results, with detectable levels of POPs found in all the samples collected.

**Discussion and Conclusion:** The study establishes a correlation between microplastics and POPs in Callao Bay, with the highest concentration at the Chillon River’s mouth. The presence of POPs in all samples indicates potential adverse effects on marine organisms and ecosystems. Urgent mitigation strategies and stricter pollution control measures are needed to address this issue, while further research is required to explore the complex relationship between microplastics and POPs in marine environments globally.

**References:**


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Introduction:
The micro- (MPs, particles smaller than 5 mm) and nanoplastics (NPs, particles smaller than 1 µm) particles emitted by tyre abrasion may impact air quality and there is raising concerns over harmful effects on human health.¹ Such tyre particles are mainly made of elastomeric polymers, fillers, vulcanization agents and additives. Among the techniques commonly used for their analysis, the thermo-analytical technique, pyrolysis gas chromatography mass spectrometry (Py-GC/MS) is an emerging powerful tool for the identification and quantification of low-µm range MPs (<10 µm) and NPs. The way to a standardized analysis of tyre particles and their quantification with Py-GC/MS has been initiated with the ISO/TS 21396:2017 protocol.² This procedure focuses on the quantification of dimeric pyrolysis fragments from styrene butadiene rubber (SBR), butadiene rubber (BR) and natural rubber (NR) using corresponding deuterated internal standards in order to ensure the reproducibility of the measurements.

The main aim of this study is the analysis of different size fractions of tyre wear particles (TWPs) produced under laboratory conditions by means of Py-GC/MS.

Materials and methods:
The tyre samples were cut in square pieces of 10 cm x 10 cm and the Taber® Abraser 5130 was used as abrasion unit. The sample was placed in Taber® Abraser, in a particle-free chamber and a total of 200 cycles were performed. The particles were collected using a mini-moudi® impactor, enabling the size fractionation of the collected TWPs on aluminium filters (10, 5.6, 3.2, 1.8, 1.0, 0.56, 0.32, and 0.18 µm pore size). The different filters were folded and directly inserted in the pyrolysis cup without prior treatment. The pyrolysis as well as the gas chromatography and mass spectrometry conditions were set as described in the ISO/TS 21396:2017.

Results:
Py-GC/MS was employed to gain insight into the chemical characterization of different size fractions of TWPs produced during abrasion tests (Figure 1) giving the average mass distribution of collected TWPs. The quantification has been done using the dimeric pyrolysis fragments including the vinylcyclohexene for butadiene rubber and styrene butadiene rubber and dipentene for natural rubber and their corresponding deuterated internal standards in order to correct the matrix effects. Calibration curves with acceptable R² were obtained enabling the quantification of the different polymers contained in the different size fractions.

Discussions and conclusions:
The obtained results showed that different sizes of particles are produced during the tyre abrasion and that the Py-GC/MS proposed in ISO/TS 21396:2017 is a suitable method to identify and quantify the emitted TWPs after abrasion of tyres.

For a future work, the developed method will be applied to examine how different types of tyres including summer, winter and four seasons tyres impact the size distribution and generation mechanism of TWPs.

Acknowledgment:
M.V. acknowledges EU funded project MS4Plastics (H2020-MSCA-IF-2020 - Grant Agreement No 101023205).

References:
1. OECD. Policies to reduce microplastics pollution in water focus textiles and tyres; Paris, 2021.
POPs in Developing Countries

P-105  Spatial distribution of halogenated polycyclic aromatic hydrocarbons in coastal area of Gyeonggi Bay, Korea

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Introduction:
Halogenated-PAHs (Hl-PAHs), encompassing chlorinated-PAHs (Cl-PAHs) and brominated PAHs (Br-PAHs), originated from incomplete combustion in the presence of specific halogen sources, such as waste incineration. These compounds exhibit both carcinogenic and mutagenic properties. Furthermore, particular homologs, including 7-monobromobenz[a]anthracene, 7-chlorobenzo[a]anthracene, 3,8-dichlorofluoranthene, 4,7-dibromobenz[a]anthracene, and 6-chlorochrysene, have been found to be more toxic than benzo[a]pyrene. The distribution of Hl-PAHs has predominantly been investigated in gaseous and particulate matter in the atmosphere, waste incineration processes, and effluent discharge from wastewater treatment facilities within freshwater environments. However, there is a scarcity of studies conducted in coastal and offshore areas.

Materials and Methods:
Sediments were collected from 19 intertidal and 57 subtidal zones in Gyeonggi Bay, South Korea. Ten Cl-PAHs and twenty Br-PAHs were analyzed utilizing Gas Chromatography-Mass Spectrometry (GC-MS).

Results:
The distribution of Cl-PAHs and Br-PAHs in the intertidal zone exhibited relatively high concentrations within Gyeonggi Bay and comparatively low concentrations outside Gyeonggi Bay. The detection rate of Cl-PAHs was 32%, identified in 6 out of 19 sites, and Br-PAHs were detected at all sites. The concentration of Cl-PAHs ranged from MDL to 60 ng/g dry weight (dw) (mean: 18 ng/g dw), while the concentration of Br-PAHs varied between 3.4 and 898 ng/g dw (mean: 108 ng/g dw). In the subtidal zone, the distribution of Hl-PAHs was elevated near ports, sewage treatment plants, and the Han River estuary. The detection rate of Cl-PAHs was 11%, found in 11 out of 57 sites, and Br-PAHs were detected at all sites. The concentration of Cl-PAHs ranged from MDL to 19 ng/g dw (mean: 5.3 ng/g dw), and that of Br-PAHs ranged from 0.6 to 284 ng/g dw (mean: 8.5 ng/g dw).

Discussion and Conclusion:
The sources of Cl-PAHs and Br-PAHs exhibited distinct trends. Upon tracing the sources by concentration, it was determined that landfills and ports served as the primary contributors to Cl-PAHs, while sewage treatment plants were the main sources of Br-PAHs. Given that Br-PAHs are distributed at relatively higher concentrations compared to Cl-PAHs in both intertidal and subtidal zones, it is postulated that sewage treatment plants situated around Gyeonggi Bay are the principal sources of contamination.
POP-P107 Persistent Organic Pollutants (POPs) in the Surroundings of Electronic Waste Recycling Sites in Chachoengsao Province, Thailand

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¹ Ecological Alert and Recovery Thailand (EARTH), 211/2, Ngamwongwan Rd. 31, Nonthaburi 11000, Thailand
² Arnika – Toxics and Waste Programme, Seifertova 85, 130 00 Praha, Czech Republic, jindrich.petrlik@arnika.org
³ International Pollutants Elimination Network (IPEN), PO Box 7256 SE-402 35, Göteborg, Sweden

1. Introduction:
Electronic waste (e-waste) and its imports from abroad represent a major burden for the environment and human health in Thailand. This study is focused on mapping pollution by POPs (Persistent Organic Pollutants) in the vicinity of two facilities processing e-waste in Chachoengsao province, and one site affected by the disposal of sludge of unknown origin (Hat Nang Kaeo) in Prachinburi province. We focused on POPs which are used as additives in electronic equipment and plastic used for its casing, such as, for example, brominated flame retardants (BFRs), short-chain chlorinated paraffins (SCCPs), and others. We also focused on POPs produced unintentionally during the production of BFRs, and particularly during incineration and other thermal processes used for the disposal and recycling of plastics from e-waste. This study also compares results presented in previous similar studies led by Arnika and EARTH and summarized in the report "Toxic Hot Spots in Thailand"¹, and in several abstracts presented at Dioxin Conferences².⁴. There is also a large number of studies looking at POP levels at sites affected by e-waste dismantling in China⁵,⁶, Vietnam⁷,⁸, and Indonesia⁹. We compared the results of the analyses in this study with these previous studies as well.

2. Materials and Methods:
For the sampling in this study, we chose two sites near factories which handle e-waste. They state their primary activity is recycling. However, the e-waste is mainly dismantled, and only metal parts are recycled in the factories. Residual waste, including plastic, is often burned in some kind of incineration operations. We took samples of soils, sediments, dust, and free-range duck eggs. All samples were collected as pooled samples composed of multiple individual samples. More details about sampling can be found in the broader report published in November 2022¹¹.

The results of the analyses for thirteen samples in total are evaluated in this study. The widest range of samples were taken in the surroundings of the Supcharoen Recycle Co. Ltd. factory in Khao Hin Son subdistrict: 2 sediment samples, 2 dust samples, 1 sample of soil, and one sample of free-range duck eggs. Soil and dust samples were also taken close to the CT Steel Co. Ltd. factory, one of the electronic waste recycling factories located in Moo 1 “Ban Muang Phrong” village, Khao Hin Son subdistrict, Phanom Sarakham district, Chachoengsao province. One sample of contaminated soil was taken at Hat Nang Kaoe site. Reference samples of dust, soil, and sediment were taken in a clean area of an organic farm in Na Somboon village, Kalasin province. A reference sample of chicken eggs was obtained in a supermarket in Maha Sarakham in February 2022.

All samples were analysed for their content of seven indicator PCB congeners (= non-dioxine-like PCBs; ndl PCBs), hexachlorobutadiene (HCBD), pentachlorobenzene (PeCB), hexachlorobenzene (HCB), 16 PBDE congeners, three HBCD isomers, six novel BFRs (nBFRs; 1,2-bis(2,4,6-tribromophenoxy) ethane (BTBPE), decabromodiphenyl ethane (DBDPE), hexabromobenzene (HBB), octabromo-1,3,3-trimethylphenyl-1-indan (OBIND), 2,3,4,5,6-pentabromobenzylbenzene (PBBEB), and pentabromotoluene (PBT))., and tetrabromobisphenol A (TBBPA). All samples except KH-S-1 were also analysed for 13 PCN congeners (PCN 4, PCN 9, PCN 18, PCN 20, PCN 41, PCN 42, PCN 52, PCN 56, PCN 66, PCN 70, PCN 73, PCN 74, and PCN 75). The analytes were extracted by a mixture of organic solvents, hexane: dichloromethane (1:1). The extracts were cleaned by means of gel permeation chromatography (GPC). The identification and quantification of the analyte were conducted by gas chromatography coupled with tandem mass spectrometry detection in electron ionization mode for the analyses of PCBs, HCBD, PeCB, HCB, and PCNs. The identification and quantification of PBDEs and nBFRs were performed using gas chromatography coupled with mass spectrometry in negative ion chemical ionization mode (GC-MS-NICI). The identification and quantification of HBCD isomers and TBBPA were performed by liquid chromatography interfaced with tandem mass spectrometry, with electrospray ionization in negative mode (UHPLC-MS/MS-ESI). Four reference samples, two sediments and duck eggs from Nong Khok, and a soil sample from Hat Nang Kaoe were transferred into cyclohexane and diluted. The identification and quantification of SCCPs were performed using gas chromatography/time-of-flight high resolution mass spectrometry (GC/TOF-HRMS) in the mode of negative chemical ionization (NCI). All of the above-mentioned analyses were conducted in a Czech-certified laboratory (University of Chemistry and Technology, Department of Food Chemistry and Analysis). All samples except the soil sample from Hat Nang Kaoe were analyzed for their content of individual PCDD/Fs, twelve dioxin-like PCB congeners, and for polybrominated dibenzo-p-dioxins and dibenzofurans (PBDD/Fs) by HRGC-HRMS in the MAS laboratory, Münster, Germany. The accredited MAS_PA002, ISO/IEC 17025:2005 method was used to determine PBDD/Fs.
The basic steps of the analyses can be summarized as follows:

- Addition of 13C12-labelled PBDD/F internal standards to the sample extract
- Multi-step chromatographic clean-up of the extract
- Addition of 13C12-labelled PBDD/F recovery standards
- HRGC/HRMS analysis

Quantification was performed according to the internal labelled PBDD/F standards (isotope dilution technique and internal standard technique).

3. Results:
Contamination with POPs was revealed at all three locations researched in this study, Nong Khok village (Moo 9) Khao Hin Son subdistrict, Moo 1 village Khao Hin Son subdistrict, and Hat Nang Kaeo subdistrict. The highest levels were observed in the surroundings of the Supcharoen Recycle Co. Ltd. factory, in the village of Nong Khok, where contamination of the food chain was confirmed by high levels of some POPs in free-range duck eggs. The dismantling and incineration of e-waste is most likely to be the source of this serious contamination. The dumping of industrial sludge from a drum “donated” by a factory to villagers caused serious contamination with SCCPs.

Table 2. Summarized results of the analyses of the samples from Nong Khok, Hat Nang Kaeo and Khao Hin Son Moo 1 sites. The results are in ng/g of dry matter for dust, soil, and sediment and in ng/g of fat for eggs respectively. PCDD/Fs, dl PCBs and PBDD/Fs in pg WHO-TEQ/g of dry matter for dust, soil, and sediment and in ng/g of fat for eggs respectively.

<table>
<thead>
<tr>
<th>Locality Khao Hin Son - Nong Khok</th>
<th>Hat Nang Kaeo</th>
<th>Khao Hin Son Moo 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample ID</td>
<td>KHS-S-1</td>
<td>SCN-D-01</td>
</tr>
<tr>
<td>Matrix</td>
<td>Soil</td>
<td>Dust</td>
</tr>
<tr>
<td>Fat content (%)</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>PCDD/Fs</td>
<td>19</td>
<td>14.6</td>
</tr>
<tr>
<td>DL PCBs</td>
<td>10.6</td>
<td>1.43</td>
</tr>
<tr>
<td>PCDD/F + dl PCBs</td>
<td>21</td>
<td>16</td>
</tr>
<tr>
<td>PBDD/Fs</td>
<td>18</td>
<td>10.2</td>
</tr>
<tr>
<td>HCB</td>
<td>&lt;0.02</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>PeCB</td>
<td>0.28</td>
<td>0.24</td>
</tr>
<tr>
<td>6 ndl PCB</td>
<td>0.19</td>
<td>0.20</td>
</tr>
<tr>
<td>7 ndl PCB</td>
<td>0.23</td>
<td>0.20</td>
</tr>
<tr>
<td>13 PCN cong.</td>
<td>NA</td>
<td>0.028</td>
</tr>
<tr>
<td>SCCPs</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>sum HBCD</td>
<td>&lt;0.75</td>
<td>&lt;0.75</td>
</tr>
<tr>
<td>PBDE 209</td>
<td>16.5</td>
<td>&lt;5.0</td>
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<tr>
<td>sum of PBDEs</td>
<td>21</td>
<td>2.7</td>
</tr>
<tr>
<td>BTBPE</td>
<td>0.48</td>
<td>&lt;0.01</td>
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<tr>
<td>DBDPE</td>
<td>52</td>
<td>&lt;10.0</td>
</tr>
<tr>
<td>HBBz</td>
<td>0.91</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>OBIND</td>
<td>&lt;0.1</td>
<td>&lt;0.10</td>
</tr>
<tr>
<td>PBEB</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>PBT</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>sum of nBFRs</td>
<td>53</td>
<td>&lt;10.0</td>
</tr>
<tr>
<td>TBBPA</td>
<td>&lt;1.5</td>
<td>&lt;1.5</td>
</tr>
</tbody>
</table>
**Figure 1**: Aerial view at the Supcharoen Recycle Co. Ltd. factory, closer view at part with technology, where residues from e-waste are incinerated. Photo by Karnt Thassanaphak, EARTH.

Table 3. Summarized results of the analyses of the reference samples from the Na Somboon organic farm (dust, soil, and sediment) and a supermarket in Maha Sarakham (chicken eggs). The results are in ng/g of dry matter for dust, soil, and sediment and in ng/g of fat for eggs, respectively. PCDD/Fs, dl PCBs and PBDD/Fs in pg WHO-TEQ/g of dry matter for dust, soil, and sediment and in ng/g of fat for eggs respectively.

<table>
<thead>
<tr>
<th>Locality</th>
<th>Na Somboon – organic farm – reference site</th>
<th>Reference egg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample ID</td>
<td>NSD-02</td>
<td>NS-S-01</td>
</tr>
<tr>
<td>Matrix</td>
<td>Dust</td>
<td>Soil</td>
</tr>
<tr>
<td>Fat content (%)</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>PCDD/Fs</td>
<td>&lt;0.63</td>
<td>&lt;0.63</td>
</tr>
<tr>
<td>dl PCBs</td>
<td>&lt;0.41</td>
<td>&lt;0.41</td>
</tr>
<tr>
<td>PCDD/F + dl PCBs</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
</tr>
<tr>
<td>PBDD/Fs</td>
<td>&lt;2.99</td>
<td>&lt;2.76</td>
</tr>
<tr>
<td>HCB</td>
<td>&lt;0.02</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>PeCB</td>
<td>&lt;0.02</td>
<td>0.037</td>
</tr>
<tr>
<td>6 ndl PCB</td>
<td>&lt;0.02</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>7 ndl PCB</td>
<td>&lt;0.02</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>13 PCN cong.</td>
<td>&lt;0.02</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>SCCPs</td>
<td>48</td>
<td>26</td>
</tr>
<tr>
<td>sum of HBCD</td>
<td>&lt;0.75</td>
<td>&lt;0.75</td>
</tr>
<tr>
<td>PBDE 209</td>
<td>&lt;5.0</td>
<td>&lt;5.0</td>
</tr>
<tr>
<td>sum of PBDES</td>
<td>&lt;5.0</td>
<td>&lt;5.0</td>
</tr>
<tr>
<td>BTBPE</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
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<td>DBDPE</td>
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<td>&lt;10.0</td>
</tr>
<tr>
<td>HBBz</td>
<td>&lt;0.01</td>
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</tr>
<tr>
<td>OBIND</td>
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</tr>
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<td>PBEB</td>
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<td>&lt;0.01</td>
</tr>
<tr>
<td>PBET</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
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<tr>
<td>sum of nBFRs</td>
<td>&lt;10.0</td>
<td>&lt;10.0</td>
</tr>
<tr>
<td>TBBPA</td>
<td>&lt;1.5</td>
<td>&lt;1.5</td>
</tr>
</tbody>
</table>
4. Discussion:

Very high levels of unintentionally produced POPs were confirmed in the free-range duck eggs from Nong Khok. The sample of free-range duck eggs from Noog Khok is important for the investigation of potential food chain contamination. The results of its analyses for POPs can be compared with the data presented in two global studies recently: one was focused on sites affected by plastic waste management and the other summarized levels of PCDD/Fs, dl PCBs, and PBDD/Fs in poultry eggs collected at various hot spots globally. The level of PCDD/Fs is the tenth-highest level ever measured in poultry eggs in Asia, and the second-highest level measured in eggs from Thailand. The level of PBDD/Fs in the eggs from Nong Khok is the sixth highest measured in free-range poultry eggs from polluted sites globally.

The level of PCDD/Fs and total TEQ level of PCDD/Fs and dl PCBs in the sample of duck eggs from Nong Khok exceed the maximum levels set in the EU by more than 24 and 14 times, respectively. Also, the levels of PCDD/Fs and PBDD/Fs in the soil samples from this locality are many times higher compared to the reference site.

Serious contamination with SCCPs was discovered at Nong Khok, as well as the Hat Nang Kaeo site, most probably because of the dumping of industrial sludge at these sites. It also resulted in a high concentration of SCCPs measured in the free-range duck eggs at Nong Khok. However, levels of SCCPs, several orders of magnitude higher, were measured in free-range eggs from e-waste sites in Guiyu and Logtang, China. A relatively high level of ndl PCBs was measured in the soil at Hat Nang Kaeo, in addition to contamination with SCCPs.

The levels of PBDD/Fs were most significant among the chemicals analyzed in the samples from Moo 1 village Khao Hin Son subdistrict, followed by PCDD/Fs, which shows that the burning of e-waste residues is the most important pathway of contamination at this locality.

The source of the contamination with POPs in the vicinity of facilities in Moo 1 and Supcharoen Recycle Co. Ltd., both in Khao Hin Son subdistrict, is poor management of e-waste accompanied by some form of incineration of plastics and other residues from this activity. E-waste dismantled in the Supcharoen Recycle Co. Ltd. facility obviously does not come from Thailand as confirmed in a study by the Basel Action Network. Therefore, it is very important to tighten international rules for transboundary movement of electronic waste and waste containing POPs including stricter Low POPs Content Levels defined within the framework of the Stockholm and Basel Conventions.

5. Conclusions:

The highest levels of POPs were observed in the surroundings of the Supcharoen Recycle Co. Ltd. factory, in the village of Nong Khok, where contamination of the food chain was confirmed by high levels of some POPs in free-range duck eggs. The dismantling and incineration of e-waste is most likely to be the source of this serious contamination. The dumping of industrial sludge from a drum "donated" by a factory to villagers caused serious contamination with SCCPs. Serious contamination with SCCPs was discovered at Nong Khok, as well as Hat Nang Kaeo, most probably because of the dumping of industrial sludge at these sites. It also resulted in a high concentration of SCCPs measured in the free-range duck eggs at Nong Khok. A relatively high level of ndl PCBs was measured in the soil at Hat Nang Kaeo, in addition to contamination with SCCPs. The levels of PBDD/Fs were most significant among the chemicals analyzed in the samples from Moo 1 village Khao Hin Son subdistrict, followed by PCDD/Fs, which shows that the burning of e-waste residues is the most important pathway of contamination at this locality. An e-waste dismantling and incineration of parts of it is most likely source of contamination with POPs at two studied sites in Khao Hin Son subdistrict, Thailand. E-waste dismantled there obviously does not come from Thailand only. Therefore, it is very important to tighten international rules for transboundary movement of electronic waste and waste containing POPs including stricter Low POPs Content Levels defined within the framework of the Stockholm and Basel Conventions.

6. Acknowledgments:

This study was conducted as a part of the following projects: "Increasing Transparency in Industrial Pollution Management through Citizen Science and EIA System Enhancement" financed by EU AID (EuropeAid 2017/389-531) and co-financed by the Transition programme of the Czech Ministry of Foreign Affairs, Global Greengrants Fund, and Thai Health Foundation. It is also part of larger study focused on plastic waste financially supported by Swedish government through IPEN.
7. References:


Introduction: Bahía Blanca is a coastal city in Argentina known for its industrial and commercial activities, including a petrochemical complex and a deep-water port. The city is surrounded by an estuary which is subject of environmental concern due to pollution resulting from inadequate management of these activities. In this study, our objective was to detect and quantify persistent organic pollutants (POPs) and microplastics (MPs) in various environmental compartments of Bahía Blanca, including sediments and water from waterways, atmospheric deposition, and urban dust.

Methods: Sediments were collected from six waterways discharging into the Bahía Blanca estuary, including sewage and industrial discharges, as well as urban (Maldonado and Napostá) and non-urban streams. Atmospheric deposition and urban dust was sampled within the Bahía Blanca city. Water of Napostá and Maldonado streams was sampled along rainy and non-rainy days. Dichloro diphenyl trichloroethane and its metabolites (DDXs), hexachlorocyclohexanes (HCHs), polybrominated diphenyl ethers (PBDEs), and polychlorinated diphenyls (PCBs) were analyzed using hexane-acetone Soxhlet extraction and clean-up on an acid silica column, and quantification were performed using gas chromatograph coupled to a triple quadrupole mass spectrometer. The MPs separation was performed by flotation with saturated NaCl solution followed by filtration of the supernatant for urban dust, river water and atmospheric deposition samples. The MPs were identified and classified with a stereoscopic magnifying glass.

Results: The levels of DDXs, HCHs, PBDEs, and PCBs varied significantly between discharges, showing the highest levels at the sewage discharge area (1.73, 0.24, 1.21, and 3.9 ng/g dry weight, respectively), followed by the urban stream’s outlets. The concentration of MPs in the urban streams ranged from 0 to 19.71 MPs/L, with an average of 6.36 MPs/L. During rainy and non-rainy days, the average estimated mass flow was 140,000 MPs/s (SD: 120,000) and 3,000 MPs/s (SD: 2,900), respectively. Urban dust levels ranged from 14.65 to 205.86 MPs/kg (mean of 60.92 MPs/kg), and MPs amount were significantly lower few hours after the rainfall. Atmospheric deposition of MPs ranged from 0 to 86.81 MPs/m2/day (mean of 18.1 MPs/m2/day). The average size of plastic particles in urban dust, watercourses, and atmospheric deposition was 1.11-1.38 mm, with no significant difference between the compartments. Fibers were the dominant shape of plastics in the three environmental compartments, with average proportions of 89%, 85%, and 55% in stream water, atmospheric deposition, and urban dust, respectively, while fragments had mean values of 9%, 11%, and 38% in the same compartments.

Discussion and Conclusion: The concentrations of POPs in the sediments of the urban streams were higher than those found in the Bahia Blanca estuary, and positively correlated with levels observed in the coasts near each discharge in previous studies. This point at these discharges as the main sources of POPs to the estuary. Analysis of plastics as POP carriers from the urban area is therefore relevant, and appropriate management could improve environmental quality in the estuary and beyond. The study revealed that the main source of plastics reaching the estuary is through surface runoff from storm water, with the plastics found in water, air, and urban dust having similar shapes and sizes. While previous studies have shown an association between POPs and plastics in the estuary, it is still unclear if the plastics found in the air, urban dust, and stream water are indeed carriers of POPs, and further research will be conducted in this direction. Overall, the study emphasizes the need for improved environmental management practices and additional research to better understand the sources and fate of these pollutants in the estuary. The presence of POPs and MPs in various environmental compartments underscores the urgency of addressing this issue to ensure the long-term sustainability of this critical ecosystem.

Acknowledgments: This research was funded by the MinCyT-CONICET Pampa Azul Project C17 and PGI 24Q111 funded by UNS granted to Andrés H. Arias.

References:
Introduction: The presence of toxic organic compounds, including persistent organic pollutants (POPs) in South Africa (SA) and the African continent is a source of concern. The historic pollution in Africa is in part due to their potential for long-range transport from other continents. The complexity of SA’s relationship with pesticides is in part due to the extensive agricultural industry, which heavily relies on the use of chemicals for pest control. Furthermore, the mining of coal and other natural resources, and the heavy industrialisation of SA, remains a major source of POPs. Despite being banned, some POPs are still in use or unintentionally produced. Therefore, the state of POPs, and their monitoring and analysis remain relevant. One of the challenges with analysing POPs arises from their low concentrations in numerous matrices. Furthermore, analysis often requires the use of high-end equipment, which presents challenges, as the developing world often lacks the full scope of analytical technology required for analysis. However, significant progress has been made in SA. The work presented in this paper highlights the experiences, challenges, advances and capabilities of SA in the analysis of POPs, highly hazardous pesticides (HHPs) and priority pollutants (PP) in different matrices.

Materials and Methods: For the broad-spectrum screening of organic pollutants, twodimensional gas chromatography with time-of-flight mass spectrometry (GC×GC-TOFMS) has been the preferred method of choice (de Vos et al., 2013), whilst confirmation work has been carried out using both GC-MS/MS and LC-MS/MS. The confirmation of priority pollutants such as polycyclic aromatic hydrocarbons (PAHs) has also been carried out using GC×GC-TOFMS. Both LC-MS/MS and GC-MS/MS have been used in parallel, and separately, for the confirmation of POPs, HHPs and PPs. Polychlorinated biphenyls (PCBs), PCDD/Fs and polybrominated diphenyl ethers (PBDEs) have been exclusively analysed and confirmed using GC-MS/MS, whilst perfluorochemicals (PFCs) have been analysed and confirmed by LC-MS/MS. Organochlorine pesticides (OCPs) and HHPs have been analysed using both techniques.

Results: Table 1: The analysis of different classes of POPs in different matrices.

<table>
<thead>
<tr>
<th>POPs class</th>
<th>Analytical system</th>
<th>Matrix</th>
</tr>
</thead>
<tbody>
<tr>
<td>OCPs</td>
<td>GC-TOFMS; GC×GC-TOFMS &amp; GC-MS/MS</td>
<td>Water; sediment; soil; fish; blood &amp; urine</td>
</tr>
<tr>
<td>PCBs, PBDEs &amp; PCDD/Fs</td>
<td>GC-TOFMS; GC×GC-TOFMS &amp; GC-MS/MS</td>
<td>Water; sediment; soil, mussel &amp; fish</td>
</tr>
<tr>
<td>PFCs</td>
<td>LC-MS/MS</td>
<td>Water; Ground water; waste effluent; sediment; mine tailings; bird eggs &amp; fish</td>
</tr>
<tr>
<td>Methoxychlor &amp; Chlorpyrifos</td>
<td>GC-TOFMS; GC×GC-TOFMS &amp; GC-MS/MS</td>
<td>Water; sediment; soil; fish; blood &amp; urine</td>
</tr>
<tr>
<td>PAHs</td>
<td>GC-TOFMS; GC×GC-TOFMS</td>
<td>Water; sediment; soil; fuel &amp; fuel products; fish; bird eggs; muscle &amp; food products</td>
</tr>
<tr>
<td>HHPs</td>
<td>GC-TOFMS; GC×GC-TOFMS &amp; GC-MS/MS &amp; LC-MS/MS</td>
<td>Fruit; blood &amp; urine</td>
</tr>
</tbody>
</table>

Discussion and Conclusion: SA has made significant progress in the analysis of POPs in a wide range of matrices relevant to SA. Previously, limitations were experienced due to the lack of more appropriate instrumentation for confirmation of some of the POPs. Samples were previously sent overseas for confirmation, which had major cost implications and also had the potential to leave the human population and environment at risk whilst waiting for results. Efforts have been made over the years to acquire more fit-for-purpose instrumentation, leading to a much broader scope of matrices and classes of POPs being analysed. Despite the major advancements in POPs analysis, there is still room for improvement. There is currently no accredited laboratory in the country providing routine testing of certain POPs such as PFDD/Fs, and SA could build on this capacity as it is now well equipped and perfectly positioned to perform these complex analyses on a more routine basis.

References

Introduction: Recently, the analysis of POPs such as PFASs has significantly evolved, particularly with the availability and use of sophisticated analytical instruments, which can perform accurate mass-high resolution mass spectrometry (AM-HRMS) and tandem mass spectrometry (MS–MS). Tandem mass spectrometry provides good sensitivity and selectivity for target analysis of PFASs; however, it neglects the presence of other organic contaminants such as emerging and legacy PFASs since it requires analyte specific information. The use of full spectrum acquisition techniques which provide accurate mass high resolution spectrometry such as LC-QTOF-MS are essential to obtain information about a large number of PFASs compounds present in various water matrices. Suspect and/or nontarget screening approaches are needed in order to detect the presence of potentially overlooked emerging PFASs. This is of high importance since these substances end up in the nearby waterbodies thereby adversely affecting the health of living organisms. Therefore, the present study investigated the presence of legacy and emerging PFASs in WWTPs, DWTPs, drinking tap, bottled and surface water samples.

Materials and Methods: Four brands of bottled water were purchased, tap, surface water, drinking water treatment plants (DWTPs) and wastewater treatment plants (WWTPs) samples were collected using a high-density polypropylene bottle from Gauteng Province, South Africa. Samples were extracted using Solid Phase Extraction (SPE) and Triple TOF used for non-target analysis of PFASs.

Results: Shown in Figure 1 is, PFPrOPrA, an example of several emerging PFASs identified with a score of 71.5%, MeFOSA, EtFOSA, FOSA, PFOI, 8:2 FTOH, PFHxI, PFMOPrA, FOET, 8:2 FTS and 10:2 FTCA were among the emerging PFASs identified. Most of the long-chain legacy PFASs were not detected in tap water and DWTP; whereas surface water was characterized by frequent detection of PFPeA, PFHpA, PFDA, PFHxS, L-PFOS, 6:2 FTS, FOSA, 6:2 FTUCA, 6:2 FTCA, 8:2 FTMAC and PFO3OA. In WWTPs, some PFASs were detected in the effluent, secondary setting tank (SST) but surprisingly not in the influent samples.

Discussion and Conclusion: Detection of emerging contaminants such as PFPrOPrA (Gen-X, a replacement for PFOS) poses a risk for end-users, as they are not monitored and have been reported to be even more toxic than the replaced long-chain PFASs. WWTPs serve as a transport of PFASs from pollution sources to surface water, DWTPs and to drinking water.

Acknowledgments: The authors are indebted to the Water Research Commission of South Africa for financing this project.

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P-113 Evaluation of Interlaboratory Study on PCDDs, PCDFs and Dioxin like PCBs in the PUF with Added Extract

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1. Introduction:
Inter-laboratory comparison is available for maintaining dioxin analytical quality/skills through testing by certified laboratories. There are 72 accredited laboratories for dioxin by MLAP (Specified Measurement Laboratory Accreditation Program) system of Ministry of Economy, Trade and Industry (METI) in Japan. But it is more important to maintain QA/QC system and evaluate quality of daily analysis data continuously. There are some official proficiency tests for dioxin analysis by JSAC (The Japan Society for Analytical Chemistry) and METI in Japan. Research Group on Ultra Trace Analyses (UTA) which is accompanying organization of Japan Environmental Measurement & Chemical Analysis Association (JEMCA) established in 2003. The UTA consists of 75 institutions, mostly private dioxin testing laboratories in 2023 and is responsible for developing the analytical potential of not only dioxins but also other trace level analysis of well known POPs in the environment. UTA carried out inter-laboratory comparison studies annually since 2003 for polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and dioxin-like polychlorinated biphenyls (DL-PCBs) about fly ash (R-6, R-9, R-14, R-17, R-18), fly ash extract (R-1) , fly ash and fly ash extract (R-10), soil (R-2, R-4, R-5, R-12, R-16), sediment (R-7, R-8, R-11, R-19), simulated drainage (R-13) and PUF with added extract (R-3, R-15). This paper summarizes the recent inter-laboratory study (R-20, FY 2022) conducted by UTA group for PCDDs, PCDFs and DL- PCBs in PUF with added extract.

2. Materials and methods:
The PUF with added extract of fly ash the twentieth comparison study (R-20) was sent to 53 laboratories. All member laboratories were asked to report all 2,3,7,8-substituted PCDD/DFs congeners, homologues and 12 DL-PCBs. A special result form was sent to all members in which, the following details were requested; 1. The analytical results obtained, including internal standard substance recovery percentage, 2. Complete analytical procedure followed and 3. SIM chromatograms of each sample. results of these studies are evaluated for median, normalized interquartile range (NIQR), coefficient of variation by Robust method (CV % rob) for each PCDDs, PCDFs and DL-PCBs. Furthermore z-score was calculated and evaluated by ISO/IEC 17043 (JIS Q 17043). Laboratories, which exceed ±3 of z-score were required cause analysis and report of corrective action.

3. Results and discussion:
The results of statistical analysis in the 20th comparison (R-20, 2022) are summarized in Table 1. It was reported totally 53 laboratories within the deadline. CV% rob in R-20 ranged from 4.0% to 8.2% for PCDDs/DFs congeners, 4.6% to 62.3 % for DL-PCBs, and 5.1% for TEQ (not indicated in the table).

Figure 1 describes the trends of CV% rob from the 1st to 20th comparison study. As our earlier report, significant differences were observed between laboratories, in particular for 1,2,3,7,8-PeCDF and 1,2,3,4,7,8-HxCDF, depending upon the capillary column that was used for the analysis. The main causes of these differences are due to co-eluting congeners in polar GC phase (SP-2331 or CP-Sil88) (ex. 1,2,3,7,8-PeCDF co-eluting 1,2,3,4,8-PeCDF, 1,2,3,4,7,8-HxCDF co-eluting 1,2,3,4,7,9-HxCDF). They have gradually increased number of laboratories to use GC columns that can separate other congeners in the analysis of 1,2,3,7,8-PeCDF and 1,2,3,4,7,8-HxCDF. (e.g. during R-20 study the use of such columns is 98% while it was only 24% during R-2). It shows the transition of the GC column used in Figure 2.
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Table 1. Statistical analysis of the 20th comparison (R-20, 2022) study results of PCDDs/PCDFs and DL-PCBs.

<table>
<thead>
<tr>
<th>PCDDs/DFs, DL-PCBs</th>
<th>MEDIAN (pg/m$^3$)</th>
<th>NIQR</th>
<th>CV(%) rob</th>
<th>MIN (pg/m$^3$)</th>
<th>MAX (pg/m$^3$)</th>
<th>AVERAGE (pg/m$^3$)</th>
<th>SD</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,7,8-TeCDD</td>
<td>0.0505</td>
<td>0.0042</td>
<td>8.22</td>
<td>0.0392</td>
<td>0.0660</td>
<td>0.0507</td>
<td>0.0053</td>
<td>53</td>
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<tr>
<td>1,2,3,7,8-PeCDD</td>
<td>0.2960</td>
<td>0.0156</td>
<td>5.26</td>
<td>0.2360</td>
<td>0.3620</td>
<td>0.2986</td>
<td>0.0214</td>
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<td>1,2,3,4,7,8-HxCDD</td>
<td>0.5160</td>
<td>0.0282</td>
<td>5.46</td>
<td>0.4150</td>
<td>0.5960</td>
<td>0.5185</td>
<td>0.0366</td>
<td>53</td>
</tr>
<tr>
<td>1,2,3,6,7,8-HxCDD</td>
<td>1.7400</td>
<td>0.1112</td>
<td>6.39</td>
<td>1.3400</td>
<td>2.0400</td>
<td>1.7309</td>
<td>0.1292</td>
<td>53</td>
</tr>
<tr>
<td>1,2,3,7,8,9-HxCDD</td>
<td>0.9240</td>
<td>0.0738</td>
<td>7.98</td>
<td>0.7710</td>
<td>1.1000</td>
<td>0.9363</td>
<td>0.0713</td>
<td>53</td>
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<td>7.6100</td>
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<td>5.2400</td>
<td>8.5700</td>
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<td>0.5159</td>
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<td>12.9000</td>
<td>10.9274</td>
<td>0.8807</td>
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<tr>
<td>2,3,7,8-TeCDF</td>
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<td>0.0193</td>
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<td>1,2,3,7,8-PeCDF *a)</td>
<td>0.1995</td>
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<td>0.2430</td>
<td>0.2004</td>
<td>0.0164</td>
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<tr>
<td>1,2,3,4,6,7,8-HpCDF* b)</td>
<td>0.2770</td>
<td>-</td>
<td>-</td>
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<tr>
<td>2,3,4,7,8-PeCDF</td>
<td>0.3310</td>
<td>0.0163</td>
<td>4.93</td>
<td>0.2430</td>
<td>0.3620</td>
<td>0.3290</td>
<td>0.0258</td>
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<tr>
<td>1,2,3,4,7,8-HxCDF *a)</td>
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<td>0.0135</td>
<td>4.64</td>
<td>0.2220</td>
<td>0.3300</td>
<td>0.2916</td>
<td>0.0189</td>
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<tr>
<td>1,2,3,4,6,7,8-HxCDF* b)</td>
<td>0.3660</td>
<td>-</td>
<td>-</td>
<td>0.3660</td>
<td>0.3660</td>
<td>0.3660</td>
<td>-</td>
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<tr>
<td>1,2,3,6,7,8-HxCDF</td>
<td>0.4040</td>
<td>0.0163</td>
<td>4.04</td>
<td>0.3130</td>
<td>0.4670</td>
<td>0.4032</td>
<td>0.0261</td>
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<td>1,2,3,7,8,9-HxCDF</td>
<td>0.0642</td>
<td>0.0043</td>
<td>6.70</td>
<td>0.0512</td>
<td>0.0897</td>
<td>0.0651</td>
<td>0.0060</td>
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<tr>
<td>2,3,4,6,7,8-HxCDF *a)</td>
<td>0.5980</td>
<td>0.0474</td>
<td>7.93</td>
<td>0.4440</td>
<td>0.7480</td>
<td>0.6054</td>
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<tr>
<td>2,3,4,6,7,8-HxCDF *b)</td>
<td>0.7400</td>
<td>0.0411</td>
<td>5.56</td>
<td>0.7000</td>
<td>0.7990</td>
<td>0.7414</td>
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<td>1,2,3,4,6,7,8-HpCDF</td>
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<td>OCDF</td>
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<td>0.0872</td>
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</tr>
<tr>
<td>3,4,'4','5'-TeCB(#81)</td>
<td>0.1500</td>
<td>0.0096</td>
<td>6.42</td>
<td>0.1060</td>
<td>0.1850</td>
<td>0.1502</td>
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<tr>
<td>3,3,'4','5'-TeCB(#77)</td>
<td>0.8660</td>
<td>0.0463</td>
<td>5.35</td>
<td>0.6100</td>
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<td>0.8695</td>
<td>0.0667</td>
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</tr>
<tr>
<td>3,3,'4','5','5'-PeCB(#126)</td>
<td>0.1960</td>
<td>0.0141</td>
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<tr>
<td>3,3,'4','5','5','5'-HxCB(#169)</td>
<td>0.0558</td>
<td>0.0026</td>
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<td>0.0448</td>
<td>0.0720</td>
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<tr>
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<td>0.0308</td>
<td>0.0019</td>
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<td>53</td>
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<td>2,3,3,4,'5'-PeCB(#105)</td>
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<td>0.0126</td>
<td>6.24</td>
<td>0.0387</td>
<td>0.3580</td>
<td>0.2016</td>
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<tr>
<td>2,3,3,4,'5','5'-PeCB(#114) *a)</td>
<td>0.0117</td>
<td>0.0067</td>
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<td>0.0361</td>
<td>0.1980</td>
<td>0.1166</td>
<td>0.0177</td>
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<td>2,3,4,4,'5','5'-PeCB(#114) *b)</td>
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<td>0.0022</td>
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<td>2,3,3,4,'5','5','5'-HxCB(#189)</td>
<td>0.0360</td>
<td>0.0224</td>
<td>62.29</td>
<td>0.0286</td>
<td>0.0738</td>
<td>0.0456</td>
<td>0.0171</td>
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</tr>
</tbody>
</table>

(*a) Separate single peak

(*b) Including co-elute congeners
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Figure 3 shows z-score exceed ±3 laboratory numbers in individual congeners (total 53 laboratories R-20 in 2022). Generally results from around 89% of the laboratories showed <±2 z-score in individual congeners data. Furthermore, reproducibility data on extraction procedure (≦30%) and injection (≦10%) showed appreciable results from many laboratories.

The trends number of laboratories whose results exceeded ±3 of z-score of at least one data in individual congeners, were 20 / 77 (total) for R-1, 27 / 83 (total) for R-2, 33 / 78 (total) for R-3, 23 / 75 (total) for R-4, 32 / 77 (total) for R-5, 20 / 77 (total) for R-6, 11 / 70 (total) for R-7, 8 / 74 (total) for R-8, 25 / 63 (total) for R-9, 27 (fly ash) and 23 (fly ash ext.) / 63 (total) for R-10, 21 / 58 (total) for R-11, 19 / 57 (total) for R-12, 13 / 54 (total) for R-13, 11 / 57 (total) for R-14, 17 / 59 (total) for R-15, 18 / 57 (total) for R-16, 22 / 58 (total) for R-17, 15 / 52 (total) for R-18, 23 / 50 (total) for R-19, 15 / 53 (total) for R-20. These trends indicate that individual laboratories maintain QA / QC systems for z-score in inter-laboratory comparison.
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Fig. 3 z-score exceed >±3 laboratory numbers in individual congeners (Total 53 laboratories R-20 in 2022).

4. References:
1. Introduction:
For the analysis of dioxins and PCBs in food, the amount of the extracted fat is a crucial parameter, not only because the lipophilic analytes are detect in fat, but also because fat is the reference parameter for indicating the levels of dioxins and PCBs in food, which are mostly given in pg/g fat. The amount of fat extracted can be important in whether or not maximum levels are exceeded. There is a high diversity of fat compounds in food. Due to the chemical composition of fat, polar and non-polar components can be differentiated. At the fat extraction of foods, the polarity of the solvent as well as the extraction method can influence type and amount of the extracted fat. Thus, the results of analyses for the same foods can vary depending on the used solvent and method. This allows different decisions about exceedances of maximum levels in food [2]. This project investigated the effect of solvents and extraction methods on the amount of extracted fat from the food matrices meat, hen’s eggs and salmon as well as their influence on the analysed levels of dioxins and PCBs in these samples.

2. Material and Methods:
Samples of meat, eggs and salmon were taken in the regular market. Meat and salmon were cut up. The shell of the eggs were removed. The material was combined into pool samples in each case. Finally, the pool samples were homogenized and dried.

Lipid extraction by glass column: The sample was mixed with sodium sulphate and diatomaceous earth. The glass column was filled with cotton wool and sea sand. The sample was transferred into the columns directly onto the sand layer and extracted with the appropriate solvent at room temperature.

Lipid extraction by Twisselmann: The sample was mixed with diatomaceous earth. The mixture was transferred into an extraction sleeve and covered with a piece of cotton wool. A round bottom flask was filled with 100 mL of the respective solvent mixture. The extraction time was 6 hours.

Lipid extraction by Accelerated solvent extraction (ASE): The sample was mixed with diatomaceous earth and transferred into an extraction cell (100 mL) equipped with two glass fibre filters, a layer of diatomaceous earth about 1 cm high and filled with diatomaceous earth for an automated extraction under high pressure and high temperature.

After extraction by glass column, Twisselmann or ASE the extracted lipid was transferred into an automatic clean-up system. Finally measurement of PCDD/F and PCBs was performed by GC-HRMS using two gas chromatographs. The first GC contained a DB-5ms column (60 m; 0.25 mm) for separation of dioxins and non-ortho PCBs and the second GC was equipped with a HT8-PCB column (60 m; 0.25 mm) to separate mono-ortho PCBs and ndl-PCBs The quantification of dioxins and PCBs was carried out by the isotope dilution method.

3. Results:
The following solvents were used for this project (sorted by increasing polarity): n-hexane, dichloromethane/cyclohexane 1:1, toluene/ethanol 9:1, toluene/ethanol 3:7. Since the composition of fat in food varies depending on the matrix, three food matrices with different lipid compositions were selected for this project: meat, hen’s eggs and salmon (table 1). The fat in meat consists of more than 90 % nonpolar lipids, mainly of triglycerides. Hen’s eggs contain about 28 % phospholipids, 66 % triglycerides and 5 % cholesterol. The fat in eggs is altogether more polar than that in meat. Salmon contains the most polar lipids with 55 %, in particular phosphatidylethanolamine 20.7 % and phosphatidylcholine 14.8 %. The fat content and the levels of dioxins and PCB in meat, egg and salmon were determined using three different extraction methods and four different solvent mixtures each (table 1). Figures 1, 2, 3 and 4 show the fat contents as well as the levels of dioxins and PCBs extracted in each food matrix using different extraction methods and solvents.
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P-114 Studies on the influence of eluant, extraction method and treated matrix on the amount of the extracted fat and the levels of dioxins and PCBs at the sample preparation

Table 1: extraction methods, solvents and matrices

<table>
<thead>
<tr>
<th>extraction methods</th>
<th>solvents</th>
<th>matrices</th>
</tr>
</thead>
<tbody>
<tr>
<td>• glass column</td>
<td>• toluene/ethanol 3:7</td>
<td>• fat contains mainly triglycerides</td>
</tr>
<tr>
<td>• normal pressure, room temperature</td>
<td>• toluene/ethanol 9:1</td>
<td>• eggs</td>
</tr>
<tr>
<td>Twisselmann extraction</td>
<td>• dichloromethane/cyclohexane 1:1</td>
<td>• fat contains ~28 % phospholipids</td>
</tr>
<tr>
<td>• normal pressure and, high temperature.</td>
<td>• n-hexane</td>
<td>• salmon</td>
</tr>
<tr>
<td>Accelerated solvent extraction (ASE)</td>
<td></td>
<td>• fat contains 55 % polar lipids</td>
</tr>
<tr>
<td>• high pressure, high temperature</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 1: fat content in meat, hen’s eggs and salmon extracted by different solvents and methods

Figure 2: levels of dioxins and PCBs in meat extracted by different methods and solvents
Figure 3: levels of dioxins und PCBs in hen’s eggs extracted by different methods and solvents

Figure 4: levels of dioxins und PCBs in salmon extracted by different methods and solvents

4. Discussion:
Lipid extraction efficiency may often be subject to wide variations, which can significantly affect the reproducibility of lipid analysis results both between and within laboratories. Randall et al. show variations of up to a factor of 3.5 for the results of extracted lipids during a collaborative study evaluating different solvents and methods for lipid extraction [1, 3]. Every food matrix has a specific lipid composition. Depending on this, various methods and solvents can be used for the fat extraction. Basically, in food there are neutral lipids, mainly triglycerides, and polar lipids like phospholipids. For extraction solvents of similar characteristics are necessary to include both the hydrophilic and non-polar interactions. Thus, a combination of a non-polar and a polar solvent should be chosen. The more polar the solvent, the more polar fat components, such as phospholipids, are additionally extracted, which increases the total amount of fat extracted.

Extraction by use of a glass column is carried out at normal pressure and room temperature. The Twisselmann method also works under normal pressure but as a long-term hot extraction, which can improve the lipid solubility and extraction efficiency. Accelerated solvent extraction (ASE) is an automated process and combines elevated pressures and temperatures above the boiling point. The procedure can be repeated several times.

A low polarity solvent such as n-hexane will remove most of the non-polar lipids, while polar lipids are removed with polar solvents such as ethanol. This effect can be seen in this project very well at the results of hen’s eggs. Hen’s eggs contain 25 % polar phospholipids. When extracted with n-hexane using glass column, the fat content is 7.43 % versus 10.04 % using toluene/ethanol 3:7. In the case of salmon, on the other hand, this effect is not so pronounced, although it contains around 55 % phospholipids. The fat content is 9.87 % when extracted with n-hexane versus 10.65 % with toluene/ethanol 3:7. Surprisingly,
there are also differences in the extraction of meat although meat contains mainly nonpolar lipids. With n-hexane 7.65 % and with toluene/ethanol 3:7 8.5 % fat is extracted. In general, it can be said that the fat yield is higher when a more polar solvent is used for extraction. Working under normal pressure and room temperature with glass column provides a less exhaustive fat extraction than working under elevated temperature and/or pressure. Interestingly, the fat yield of Twisselmann extraction with elevated temperature but under normal pressure was slightly higher than ASE with elevated pressure and temperature. For all matrices, there is a slight advantage for extraction according to Twisselmann. One reason for this could be the long extraction time with many runs. Due to this, Twisselmann extraction seems to be more effective than extraction using ASE, although this works under increased pressure and temperature. Haedrich et al. compared the extraction efficiency of a modified SMEDES extraction (MSE) with the Twisselmann extraction and found that the amount of the extracted lipids in cows whole milk and bovine meat were comparably with both methods. In samples with fat components more polar, like hens eggs, bovine liver and fish meat, the extraction efficiency by MSE was significantly higher than with Twisselmann [1].

Furthermore, the project observed to what extent the levels of dioxins and PCBs change when different solvents and extraction methods are used. Since fat is the reference parameter, an influence is assumed here. The levels of dioxins, dl-PCBs and ndl-PCBs in the meat are in a very low range (Figure 2). The extraction method and the solvent used have only a minor influence on the extracted levels of dioxins, dl-PCBs and ndl-PCBs. With increasing polarity of the solvent, the analysed dioxin and PCB contents decrease slightly. This could be explained by the fact that more fat was extracted. Surprisingly, in the case of hen’s eggs, neither the solvent nor the extraction method had a clear influence on the levels of dioxins, dl-PCBs and ndl-PCBs analysed although the fat contents showed a dependency on the solvent used (Figure 3). However, extraction according to Twisselmann seems to have a favourable influence on the extraction results. Figure 4 shows the results of the determination of dioxins, dl-PCB and ndl-PCB depending on the solvent and the extraction method in salmon. For fish, the dioxin and PCB levels are normally not related to fat but to wet weight. For a better comparability and in particular to consider the analytical influence of the extracted fat content, the reference value of fat was also chosen for this project. The levels of dioxins in salmon are very low. The measured dl-PCB levels are elevated. Also for salmon, no significant differences between the results using the different solvents and extraction methods were detectable.

5. Conclusion:
Due to the fact that levels of dioxins are given in µg/g fat, the fat extraction is of particular importance. Dioxins and PCBs can be extracted well with all solvents. Nevertheless, slight differences were observed in the project. These correlate with the extracted fat content. The more fat is extracted, the lower the dioxin and PCB content. For this reason, the selection of the right solvent and an appropriate extraction method depending on the specific food matrix is important, especially for the decision whether the food exceeds maximum levels or not.

6. References:
**Introduction:** Per- and polyfluorinated alkyl substances (PFAS) products have been in use for more than 60 years. Research has revealed the high toxicity of PFAS compounds and thus the resulting need to regulate these substances. Therefore, the analytical interest in these compounds has increased rapidly in the last few years. The current and upcoming regulations makes it necessary to test environmental samples from various locations for PFAS content. The US EPA has published a draft method for environmental samples: 1633 (3rd draft). The method requires an elaborate extraction process, followed by a solid phase extraction (SPE) step and an additional dispersive carbon clean-up step. This work shows a streamlined sample preparation process. A new single SPE cartridge solution with a PFAS enrichment optimised polymeric sorbent is presented.

**Materials and Methods:** The full workflow of PFAS sample preparation in environmental samples is presented. Extractions of analytes from solid samples via different methods were conducted. For the enrichment and/or purification of PFAS compounds solid phase extraction (SPE) a single cartridge solution was used. The EluCLEAN® PFAS – WAX/GCB SPE column contains 150 mg of a weak anion exchanger, mixed-mode polymeric sorbent mixed with 10 mg of graphitized carbon black. For the critical evaporation step a vacuum centrifuge with cold trap named D-EVA was used. The samples were subsequently analysed by LC-MS/MS.

**Results:** An appropriate extraction method together with the blind value free robotic system FREESTYLE in combination with the new EluCLEAN® PFAS – WAX/GCB cartridges show excellent reproducibility, high recoveries and low standard deviations. The right evaporation method ensures no loss of volatile and long chain PFAS in the final sample preparation step.

**Discussion and Conclusion:** The presented workflow shows a streamlined sample preparation process with extraction and automated sample preparation for PFAS analysis in soil. Further, the single SPE cartridge solution is ideally suited for SPE of PFAS from soil and other environmental matrices. It can equivalently replace the dispersive clean-up step + WAX SPE cartridge used in US EPA 1633 (3rd draft). The single cartridge solution saves time and costs in PFAS analysis.
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P-116  Characteristics of Polychlorinated Naphtalenes in Emission Source in Korea

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Korea Environment Corporation, 42 hwangyeong-ro, seo-gu, Incheon, 22089, Rep. of Korea

Introduction:
In 2015, polychlorinated naphthalene (below PCNs) became a Stockholm Convention document and was listed as Annex A (Eradication) and Annex C (Unintentional Production). However, there were not many studies related to PCNs included in exhaust gas. Accordingly, this study aims to analyze the behavior of PCNs among the exhaust gases emitted from current incineration facilities.

Material and Method:
This study was conducted based on US EPA Method 1613. As the sample collection site, a facility that incinerates industrial waste and domestic waste was selected. Samples collected from the exhaust gas before and after the prevention facility of the incinerator were extracted by the Soxhlet extraction method, and after injecting an internal standard solution for purification into the extract solution, it was purified and analyzed by HRGC/HRMS.

Result:
As a result of comparing homologues of PCNs contained in the exhaust gas before and after the prevention facility, the average concentration reduction rate is 86.74%. Concentrations of each congener were similar in industrial waste incineration facilities and domestic waste incineration facilities. And the concentration ratio of each homologue of PCNs was higher in the order of Mono-CNs > Di-CNs > Tri-CNs > Tetra-CNs > Penta-CNs > Hexa-CNs > Hepta-CNs > Octa-CNs.

Discussion and Conclusion:
The concentrations of PCNs analyzed in industrial waste and domestic waste incineration facilities were similar to each other, so no correlation could be found according to the type of waste. However, because of the small sample size, continuous monitoring is necessary in the future. The PCNs reduction rate of the prevention facility installed in the waste incineration plant was 86.74%, confirming that the existing prevention facility could effectively reduce PCNs in exhaust gas. It is judged that it is necessary to investigate the amount of emission from the emission source of the incineration facility.

Reference:
2. US EPA Method 1613 "Tetra- through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS"(1994)
Progress in Methods for POPs Analysis

P-117  Quantification of PCDD/DFs, DL-PCBs, and PCNs in Fly Ash using a Combined POPs APGC-MS/MS Analysis Method

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1 Wellington Laboratories Inc., 345 Southgate Drive, Guelph N1G 3M5, Canada
2 Waters Corporation, 34 Maple Street, Milford 01757, U.S.A

Introduction: Fly ash produced during municipal waste incineration typically contains high levels of persistent organic pollutants (POPs) such as polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), dioxin-like polychlorinated biphenyls (DL-PCBs), and polychlorinated naphthalenes (PCNs) (collectively referred to as DFPCNs)1,2. As the use of waste incineration increases with growing populations, proper management, disposal, and monitoring of these pollutants becomes increasingly vital. Using an in-house combined POPs sample preparation and analysis method, we calculated the DFPCN concentration and tentative TEQ for a fly ash certified reference material (CRM) with reported PCDD/PCDF certified reference values (CRVs).

Materials and Methods: Sample extraction and cleanup were carried out on lab blanks (clean baked sand), spiked blanks (fortified blank baked sand), and fly ash CRM BCR-490 (IRMM) using an in-house method modified from EPA Method 1613B. Approximately 0.5g of sample was spiked with DFPCN-ES (Wellington Laboratories Inc.) and sonicated in a 2M HCl solution for 2 hours. The acid mixture was then filtered into a separatory funnel and the POPs were extracted into DCM. The DCM extract was evaporated to near dryness and the resulting round bottom flask was utilized in the Soxhlet extraction of the fly ash sample/filter with 90:10 toluene/acetone for 16-20 hours. Extract cleanup included H2SO4 acid treatment, sulfur removal with activated copper, a multi-layer silica column, and a PX-21 carbon column. DFPCN-IS (Wellington Laboratories Inc.) was added to the final extract prior to APGC TQXS MS/MS (Waters Corp.) analysis. Tentative PCN TEQs were calculated using RPFs3.

Results: CRM BCR-490 and spiked blank samples were used to evaluate the accuracy of the DFPCN sample preparation and instrumental analysis methods. As seen in Figure 1, our preliminary results show that all target PCDD/PCDF concentrations reported for BCR-490 and all DFPCN concentrations reported in the spiked blanks were within ±20% of the CRVs and target values, except for 4 analytes which were within ±30%. Average 13C-labelled surrogate recoveries ranged between 45 and 86%. The PCDD/PCDFs made up 88% of total DFPCN concentration (116 ppb), followed by DL-PCNs (9%), and DL-PCBs (3%). The total TEQ was calculated to be 3,540 pg TEQ/g with 97% contribution from PCDDs/PCDFs, 1.8% from DL-PCBs, and 0.6% from DL-PCNs.

Discussion and Conclusion: All PCDDs/PCDFs with CRVs were accurately reported in the CRM and spiked blanks using the combined methodology. Since no CRVs were reported for the DL-PCBs and PCNs in BCR-490, we evaluated method accuracy by confirming that measured analyte concentrations in the spiked blank were within ±20% of the CRVs and target values, except for 4 analytes which were within ±30%. Average 13C-labelled surrogate recoveries ranged between 45 and 86%. The PCDD/PCDFs made up 88% of total DFPCN concentration (116 ppb), followed by DL-PCNs (9%), and DL-PCBs (3%). The total TEQ was calculated to be 3,540 pg TEQ/g with 97% contribution from PCDDs/PCDFs, 1.8% from DL-PCBs, and 0.6% from DL-PCNs.

References:
3. Food Safety Authority of Ireland (IFSA), 2010, Monitoring and Surveillance Series.
Fluctuating due to the very low concentration levels present in the solvents. In many cases not all compounds were detected in all three replicates and the concentrations measured in the replicates were ∼-HCH. These two compounds are present in all four different solvents. The compound with highest detection frequency was ∼-, ∼-, and ∼-HCH, and Oxychlordane. In acetone only two compounds were detected (Figure 4 and Table 4), namely PCB 28, and 118 and 153 in n-hexane. In DCM 8 different compounds could be detected (Figure 3 and Table 3): the PCBs 28, 52, and 149, HCB, ∼-HCH. In addition, PCB 180, Aldrin, ∼-HCH, and Heptachlorepoxide were detected in isooctane and OCS, and the PCBs -DDE, -DDE, ∼-HCH, and acetone.

3 Results:
In isooctane 12 different compounds were detected (Figure 1 and Table 1), whereas there were 11 compounds detected in n-hexane (Figure 2 and Table 2). Eight components were present in both solvents: the PCBs 28, 52, 101, 149 and 153, p,p'-DDE, HCB, and -HCH. In addition, PCB 180, Aldrin, -HCH, and Heptachlorepoxide were detected in isooctane and OCS, and the PCBs 118 and 153 in n-hexane. In DCM 8 different compounds could be detected (Figure 3 and Table 3): the PCBs 28, 52, and 149, HCB, -CH, -CH, and -HCH, and Oxychlordane. In acetone only two compounds were detected (Figure 4 and Table 4), namely PCB 28, and -HCH. These two compounds are present in all four different solvents. The compound with highest detection frequency was PCB 28 (33 detects out of 48 possible), while the compound found in highest concentrations was HCB (up to 23 fg/µL). However, in many cases not all compounds were detected in all three replicates and the concentrations measured in the replicates were fluctuating due to the very low concentration levels present in the solvents.
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P-118  Investigation of background contribution from different solvent brands used for sample preparation and analysis of persistent organic pollutants

Table 1: Number of detects in isooctane. Solvent samples were analysed in triplicates.

<table>
<thead>
<tr>
<th></th>
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<td>PCB 149</td>
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<td>PCB 180</td>
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<td>p,p’-DDE</td>
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<td></td>
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<tr>
<td>HCB</td>
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<td></td>
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</tr>
<tr>
<td>Aldrin</td>
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<tr>
<td>γ-HCH</td>
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<td>1</td>
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<tr>
<td>β-HCH</td>
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<td>cis-Heptachlorepoxide</td>
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Figure 1: Average concentrations of individual POPs detected in isooctane (fg/µL).

Table 2: Number of detects in n-hexane. Solvent samples were analysed in triplicates.

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<td>PCB 149</td>
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<td>PCB 153</td>
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<td>γ-HCH</td>
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**Progress in Methods for POPs Analysis**

P-118  Investigation of background contribution from different solvent brands used for sample preparation and analysis of persistent organic pollutants

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**Figure 2:** Average concentrations of individual POPs detected in n-hexane (fg/µL).

**Table 3:** Number of detects in dichloromethane. Solvent samples were analysed in triplicates.

<table>
<thead>
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<td>HCB</td>
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<td>β-HCH</td>
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<tr>
<td>Oxychlordane</td>
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</table>

**Figure 3:** Average concentrations of individual POPs detected in dichloromethane (fg/µL).
Discussion and conclusions

The solvents from both analysed brands have some background contamination. There are some differences between the two brands, depending on the solvent and the compounds, but the differences are very small. Thus, it is not possible to give a clear recommendation for preferences on using one solvent brand over the other.

As mentioned earlier, we used an external calibration curve, which may be a limitation for calculating the resulting concentrations. But this was an elaborate decision in order to prevent introducing possible and minor background contribution coming from impurities of the isotope labelled standards, to avoid misinterpretation/false positive detects.

Anyway, the results indicate that there are some POPs present in the organic solvents tested in this study and that solvents have to be chosen carefully also in relation to the compounds that are going to be analyzed.

In the present study only one batch of solvents was investigated for background contamination. In the future, an additional check of the batch to batch variability is recommended, which will give a better indication of variability in background contamination. In order to increase accuracy of POPs measurements in solvents, repetitive measurements with several replicates should be done with higher volumes of solvents.

To our best knowledge there are no previous published studies focusing on concentration levels of POPs in different brands of organic solvents used for extraction and sample preparation. This information is of interest for especially those who analyze legacy POPs at very low concentration levels. And since the compounds analyzed are mostly banned or restricted in use, the concentrations in human and environmental samples will drop further over the next years. Therefore, it is and will be still more crucial to have a very low background contribution from solvents during sample preparation and analysis to get adequate detection limits.

Acknowledgments

Many thanks to the Northern Norway Regional Health Authority (Helse Nord) and the Department of Laboratory Medicine, University Hospital of North Norway, for funding this work.

References

Progress in Methods for POPs Analysis

P-119  Determination of PCDD/Fs by Gas Chromatography-Triple Quadrupole Mass Spectrometry(GC-MS/MS) and High-Resolution Gas Chromatography coupled to High-Resolution Mass Spectrometry(HRGC-HRMS)

JM. Cho, G.J. Park, YM. Jeong, C.J. Lee, SE. Jeon  
Korean Environmental Corporation, 42, Hwangyeong-ro, Seo-gu, Incheon, 22689, Republic of Korea

The standard reference method for determination of polychlorinated dibenzo-p-dioxins and furans(PCDD/Fs) in environmental samples is based on the use of high resolution high resolution gas chromatography coupled to high resolution mass spectrometry(HRGC-HRMS). Although the method has high sensitivity and selectivity, it is not easily accessible in general laboratories because of significant investment, high operating costs and difficult usage. Recently, gas chromatography-triple quadrupole mass spectrometry(GC-MS/MS) has been used to determine PCDD/Fs in different complex samples as an alternative method, and this technique has provided promising results. In Korea, analysis method using only HRGC/HRMS was approved for determination of PCDD/Fs in environmental samples. However the United States Environmental Protection Agency(US EPA) provides general guidance for the method of detection and quantitative measurement of PCDD/Fs in environmental samples(water, soil, fly ash, chemical waste) using GC-MS/MS(Method 8280B). In this study, a comparative study using both GC-MS/MS and HRGC/HRMS was performed by analyzing certified reference materials(CRMs, fly ash) in order to propose the GC-MS/MS technique for quantification of PCDD/Fs in environmental samples.

Extraction and clean-up procedures of CRMs were performed by Korean Environmental Standard(ES) Methods. All standard solutions of PCDD/F congeners, including EPA-1613 STOCK(Native Stock Solution), EPA-1613 LCS(13C12-labelled compound stock solution), and EPA-1613 CVS(calibration and verification solutions, CS1 to CS5) were purchased from Wellington Laboratories Inc. GC-MS/MS and HRGC-HRMS analyses were performed using a 7000D GC/TQ(Agilent Technologies, USA) and JMS-800D Ultra Focus(JEOL, Japan), respectively. Electron Impact ionization(EI) was applied for the ionization mode and analytes were separated by a VF-Xms column(Agilent Technologies, USA). After confirmation of retention times and mass spectra of the target compounds in the scan mode, quantitative analysis was performed in modes of selected ion monitoring(SIM) and multiple reaction monitoring(MRM). The collision energies and transitions from different precursors to product ions were optimized to achieve high sensitivity and selectivity in order to determine of PCDD/Fs using GC-MS/MS. Averaged relative response factors(RRFs) were used to calculate the concentrations of the target compounds on both HRGC-HRMS and GC-MS/MS.

Quality criteria including linearity, selectivity and recovery were investigated for HRGC/HRMS and GC-MS/MS. Linearity tests were optimized and applied to five calibration standard solutions including native and 13C12-labelled TCDD/Fs. The determination coefficient(R2) values higher than 0.999. The average relative response factor(RRF) was obtained by averaging the RRFs calculated for five points on the calibration curve in each series of standards. The RRF values for the PCDD/Fs had relative standard deviation values(RSD%)<15% for both methods and were consistent with the criteria established in the Korean ES method. We evaluated the analytical efficiency to assess the accuracy of the validation tests using CRMs(Fig. 1). The accuracy resulted higher than 75% for the certified congeners and there was a good agreement and met the criteria of the Korean ES method.

In this study, the method for determination of PCDD/Fs by GC-MS/MS was developed and comparative results between the reference method(HRMS) and GC-MS/MS technique was meaningful to assess the applicability for alternative method. From the result of this study, more studies are required to be introduced as a standard method using GC-MS/MS for monitoring of PCDD/Fs in environmental samples.
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P-120  Helium Savings and Method Optimization for the Analysis of Dioxin/Furans and other POPs with Magnetic Sector GC-HRMS using a Helium Saver solution

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Introduction: Helium shortage is a global concern, affecting laboratories and operational costs. Analytical methods need to have solutions to avoid the challenges caused by helium shortage. Magnetic Sector High Resolution GC/MS is the golden standard for high sensitivity analysis of Dioxins and other POPs. Already for decades it has been proving its proficiency in this field of analysis and thus became the established analysis technique available nowadays in leading Dioxin laboratories throughout the world. Using the helium saving option, the helium consumption can be reduced drastically by substituting all GC flows except the flow in the analytical column by nitrogen.

Materials and Methods: Instrument method optimization for Dioxin and Furans, PBDE and PCBs of the helium saving option. Direct comparison between two GCs attached to one Thermo Scientific DFS Magnetic Sector mass spectrometer with only one GC equipped with the helium saving option.

Results: Performance parameter such as sensitivity, peak shape and chromatographically resolution are similar between the GC equipped with the helium saving option towards the GC without this option. The Helium consumption can be drastically reduced using the Helium in a typically POPs analysis compared to a standard GC setup.

Discussion and Conclusion: The Helium saving option can be applied to POPs applications following official methods such as EPA 1613 method, EPA 1614 method, EPA 1668 method.

References: EPA 1613 method, EPA 1614 method, EPA 1668 method
Progress in Methods for POPs Analysis

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Introduction: Until the last decade, chemical monitoring in environmental, food and human matrices relied on selective and sensitive targeted screening (TS) strategies, based notably on hyphenated techniques combining chromatography and mass spectrometry. Taking advantage of recent advances in chromatography and high resolution mass spectrometry (HRMS), less selective strategies have emerged in the last decade, through suspected and non-targeted screening (NTS), to open the scope to the simultaneous analysis of suspected and unsuspected chemicals, even the identification of unknown chemicals. This study explored the complementarity between TS and NTS based on liquid and gas-phase chromatography coupled to (high-resolution) mass spectrometry (LC-/GC-(HR)MS) for the comprehensive characterization of organohalogen fingerprints within a set of Lake Ontario lake trout samples.

Materials and Methods: Ten samples of lake trout (whole fish homogenates) were collected in 2018 from the western basin of Lake Ontario at Niagara on the Lake, Ontario, Canada. The concentrations of >100 legacy, emerging and novel halogenated compounds (HCs), were determined through 4 TS approaches involving 6 hyphenated systems as follows: (i) Thirty-six BFRs were analyzed using GC/EI/HRMS, GC/APCI+/MS/MS, LC/ESI-/HRMS and LC/ESI-/MSMS platforms. (ii) Eighteen PCBs and 17 PCDD/Fs were analyzed using GC-HRMS, (iii) OCPs were measured by GC-MS/MS and (iv) CPs were analyzed using LC-HRMS. In parallel, an NTS strategy, involving both LC and GC-Q-Orbitrap, was implemented to specifically highlight halogenated signals. Non-targeted HRMS data were processed under the HaloSeeker software based on Cl and Br isotopic ratio and mass defect.

Results: TS detected 125 HCs (25 BFRs, 55 CP homologue groups, 8 OCPs, 17 PCBs and 17 PCDD/Fs) in fish samples. PBDEs (−28/-47/-49/-99/-100/-153/-154/-183), PBBs (−52/-101/-153), ∼-/∼-HBCDD, dieldrin, ∼-chlordane, Mirex, p-p’-DDD, p-p’-DDE, p-p’-DDT and all the PCBs/dioxins/furans congeners were systematically detected in all the samples. Emerging (TBBPA) and novel (nHBB, nPBBand TriBRPs) BFRs were also detected in all the fish samples. MCCPs then LCCP_L%Cl and SCCP_L%Cl were the most frequently detected groups of CPs (i.e., detection frequency = 90–100%) while LCCP_H%Cl were never detected. NTS detected 91 HC compounds. The combination of TS and NTS strategies allowed detecting a total of 195 halogenated molecules in the lake trout homogenate samples. Among the 125 halogenated molecules measured by TS, only 21 were also highlighted by NTS, mainly PCB and PBDE congeners, DDT/DDE/DDD, as well as HBCDD (∼-, ∼-) and tri-BRP.

Discussion and Conclusion: The LC/GC proportion of amenable molecules was around 50/50, confirming their complementarity for the comprehensive characterization of environmental pollutants. Using the NTS approach, halogenated poly-aromatic compounds (e.g., PCBs, PBDEs, DDTs) were only highlighted by GC while LC allowed specific identification of phenols and heavy brominated flame retardants. While the specific and sensitive TS approaches are essential for detecting and quantifying trace levels (pg g−1) of known HCs, NTS approaches undeniably expanded the characterization to unsuspected HCs, and even to proposed new C10-C14 organohalogen formulations. The possibility of substituting the many TS methods with only two NTS approaches to reduce the length of analysis, sample size and cost is very attractive in theory, but this study shows the current limitations.

Acknowledgments: French Région Pays de la Loire through the "Recherche-Formation-Innovation: Cap-Aliment Food 4 Tomorrow (RFI-Food 4.2)” program (Grant FISHCONTAM).

References:
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**Introduction:** Different class of PFAS and different matrices demand different needs for extraction, enrichment and clean-up. In water, the SPE of short chain and longer chain PFAS can be very demanding. Volatility of certain PFAS compound could also be a problem during the sample preparation [1]. Additionally, solid samples (especially soil samples) have often the ability to strongly bind neutral and long chain PFAS. An appropriate method for extraction of these PFAS from solids and optimization of critical evaporation step has to be established. Further, more demanding sample matrices, for instance, food/feed matrices are handled with a more elaborate Dual-SPE or a combination of SPE and dispersive clean-up to remove matrix interferences. In spite of the tedious process, all of them lack the ability to enrich some specific PFAS in a sufficient way e.g. neutral sulfonamides or long chain PFAS. The challenge is to develop a SPE solution that is not cost intensive and at the same time effective for the clean-up and enrichment for all types of PFAS analytes.

**Materials and Methods:** Different extraction methods were used. For the enrichment and/or purification of an extended range of PFAS compounds solid phase extraction (SPE) new SPE cartridges were developed.

**Results:** An appropriate extraction method for an extended range of PFAS including long chain PFAS was established. The newly developed SPE cartridges show excellent reproducibility, high recoveries and low standard deviations for an extended range of PFAS analytes in different matrices.

**Figure 1:** Recovery rates in % of 54 PFAS analytes in drinking water using EluCLEAN® PFAS - Universal, n =3, conc. 0.325 - 40 ng in 50 mL tap water

**Discussion and Conclusion:** Extraction methods and SPE cartridges with a superior performance for enrichment and clean-up of an extended range PFAS from drinking water, food matrices and environmental samples were developed.

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P-123  Simultaneously Clean Up of PCDD/F and all 209 PCBs, completely separated in 2 fractions

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**Introduction:**
The list of the Stockholm Convention of persistent organic pollutants (POPs) for elimination or restricted use grows constantly. Therefore, many labs are facing the challenge to extend their scope of analytical methods. To free up the capacities required for this purpose, one possible strategy is to optimize existing processes by combining established methods. There are well-known methods for the analysis of the 2,3,7,8 substituted polychlorinated dibenzo-para-dioxins and -furans (PCDD/F) and polychlorinated biphenyls (PCB), especially the US-EPA Method 1668C for all 209 PCBs, the US-EPA Method 1613 for the 2,3,7,8 substituted PCDD/F and the Commission Regulation (EU) 2017/644 for the 2,3,7,8 substituted PCDD/Fs, dioxin-like PCBs and indicator PCBs.

This poster describes a method to purify sample extracts for the analysis of the 2,3,7,8 substituted PCDD/Fs and all 209 PCBs in one single clean-up, separated in two fractions. This helps laboratories to save solvents, sorbents and time.

A three-column setup is selected for this automated clean-up.

An acidic multilayer silica column is recommended as first column, but some matrices are influencing the retention time of the PCBs on the multilayer column, depending on their grade of chlorination. The acidic silica column for 209 PCB from LCTech is optimized to compensate this effect.

As a second column, the US-EPA1668 recommends a Florisil column for the 209 PCB, but there are a lot of interferences that cannot be trapped on a Florisil and not be separated from the PCB. These interferences are eluted together with the PCB into one fraction. PCDD/F and PCB can be trapped on an alumina column, while the interferences can be washed into the waste. The alumina column is also used to separate the PCB from the PCDD/F in two different fractions.

The new method has to fulfill the acceptance criteria of the US-EPA 1668C, Table 6, and the Commission Regulation (EU) 2017/644 chapter 5.6.

**Method and Material:**
As exemplary matrices for food/feed samples, 3 g crude fish oil and 3 g olive oil, each diluted in n-hexane, have been fortified with all 209 native PCBs and directly cleaned on a DEXTech Pure (LCTech GmbH). As an environmental matrix, 5 g soil has been extracted with 2 cycles toluene with the X-TRACTION system (LCTech GmbH). The evaporation of the extracts are performed within 40 min with the D-EVA system to near dryness and re-dissolved in 10 mL n-hexane before the clean-up with the DEXTech Pure system. All samples are spiked with PCB-LCS-H (Wellington Laboratories) and EDF5525x100 (CIL). As column setup for the DEXTech Pure an acidic silica column for 209 PCB as first column, an alumina column as second column and a modified carbon column as third column are used. As solvents, n-hexane is used for sample loading, elution of the first column and the transfer onto the alumina column. With 24 mL toluene, the PCBs are collected in the first fraction. This fraction is evaporated to 200 µL via the D-EVA and spiked with PCB-ISS-H (Wellington Laboratories). The remaining PCDD/F on the alumina column are transferred on the carbon column with n-hexane/dichloromethane. With 10 mL toluene the PCDD/F are eluted from the carbon column as second fraction. This fraction is evaporated to near dryness and spiked with EDF-5526x100 (CIL).

The process time of 50 min for the clean-up includes loading and conditioning, with a total solvent consumption of 218 mL. The clean extracts of the PCB fraction are measured with the HRGC-HRMS DFS (Thermo Fisher Scientific), equipped with a SGE HT8-PCB (Trajan). For the PCDD/F fraction the RTX-Dioxin2 (Restek) has been used.

**Results and Discussion:**
All acceptance criteria of the US-EPA 1668C Table 6. are fulfilled for the 13C-recoveries. The fortified PCBs are recovered with high precision. The chromatograms of the automated clean-ups are showing low noise and no significant disturbances. The fortified level of the 209 PCB is recovered with high precision. The acceptance criteria of the Commission Regulation (EU) 2017/644 chapter 5.6 are fulfilled for the 13C-recoveries of the PCDD/F.

The difficult sample preparation for all 209 PCBs can easily be done with the X-TRACTION system and the automated clean-up systems DEXTech Pure and DEXTech 16. The column setup can be used for environmental and food/feed samples. Finally, a smooth evaporation to reduce losses of the highly volatile mono- and di-chlorinated PCB is no challenge for the D-EVA.
Introduction: Metal-organic frameworks (MOFs) are a class of hybrid organic-inorganic materials based on the assembly of organic linkers and metal ions to form crystalline networks with high pore volumes and surface areas, good thermal and chemical stability. This class of materials has recently attracted wide attention in the sample preparation field focusing on various applications of MOFs as advanced sorbent and/or sensor materials. MOFs consisting of aromatic ligands and d-metals are effective sorbents for solid-phase extraction (SPE) of PAHs and PCBs from the environmental samples at low concentration levels. The selectivity of the sorbents is high due to realizing of adsorption mechanisms based on unspecific as well as specific interactions: the hydrophobicity and dipole–dipole interaction, π–π stacking, chelation between metal ions and Cl-substituents. At the same time, their possible application for dioxins extraction has not been widely studied. Applying of MOF as SPE sorbents is an environmentally friendly approach, as it can significantly reduce the consumption of organic solvents. In addition, because of due to their selectivity and stability, the MOF consumption is low, and these sorbents are recyclable. In this work, we investigated the use of the MOFs consisting of zinc and iron ions with aromatic carboxylic acids for the extraction of polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) from the environmental water samples.

Materials and Methods: The complexes of Fe(III) ions with 1,3,5-benzenetricarboxylic acid (Fe-MOF) and Zn(II) ions with 1,3,5-tris(4-carboxyphenyl)benzene (Zn-MOF) were tested as SPE sorbents for PCDDs/Fs and PCBs analysis. The sorbents (150.0 ± 0.1 mg) were tightly pressed in a polypropylene cartridge between two PTFE frits. The cartridge prepared this way was placed in a vacuum manifold. A water sample was spiked with the solution of 13C-labelled internal standards in tetrahydrofuran, mixed on the shaker to equilibrate, and passed through the cartridge under vacuum at a rate of 1 drop per 2-3 seconds. Then, the cartridge was dried under a vacuum, and the analytes were eluted with acetone and toluene. The resulting solution was passed through a paper filter with a layer of sodium sulfate. The solvent volume was reduced to a few milliliters, transferred to a vial, and evaporated to dryness at a nitrogen stream. PCDDs and PCDFs (17 contaminants), dioxin-like PCBs (12 analytes) and non-dioxin-like PCBs (6 compounds) were analyzed with isotope dilution technique using gas chromatography coupled to high-resolution mass spectrometry (GC-HRMS).

Results: The complete method was validated by testing spiked water samples and applied to the analysis of environmental water and wastewater samples containing negligible suspended solids. The accuracy and precision of the method developed based on Zn-MOF were 11.4% and 8.9% for WHO PCDD/F-TEQ, 6.3% and 4.1% for WHO PCB-TEQ, 8.4% and 5.2% for the sum of non-dioxin-like PCBs (PCB 28, 52, 101, 138, 153, 180), respectively. The method accuracy and precision were 7.2% and 3.6% for WHO PCDD/F-TEQ, 8.5% and 3.1% for WHO PCB-TEQ, 12.0% and 6.5% for the sum of non-dioxin-like PCBs (PCB 28, 52, 101, 138, 153, 180), respectively, when applying Fe-MOF sorbent. The limits of detection are in the range 4-15 pg/L for PCDD/F.

It was found that the extraction efficiency of the analytes PCDDs/PCDFs and PCBs is different for Zn-MOF and Fe-MOF. Thus, the extraction coefficients of PCDDs and PCDFs are higher in the case of iron Fe-MOF on 3.7-12.5% than in case of Zn-MOF. At the same time, the extraction coefficients of PCBs are significant higher (18.3-34.1%) for Zn-MOF compared to Fe-MOF.

Discussion and Conclusion: The results demonstrated that the Zn-MOF and Fe-MOF are effective sorbents for solid-phase extraction of PCDDs, PCDFs and PCBs from water samples. The developed method can be used both for the analysis of water samples with a mass fraction of suspended solids less than 1%, and for samples of environmental and waste waters with a high content of sediments, after their separation and analysis separately. The different sorption properties of the MOFs can be explained both by the larger surface area of Zn-MOF (3481 m2/g compared to 427.2 m2/g for Fe-MOF).2 The selectivity to PCBs of Zn-MOF is probably the result of the presence of biaryl fragments in the bridging ligand.

References:
Introduction:
Polychlorinated dibenzo-p-dioxins/furans (PCDD/F) and their severe toxicity have been a global issue for over six decades. Although not intentionally produced, these chemicals can be formed through industrial/thermal processing of materials containing chlorinated organic compounds. These chemicals are globally restricted as they are classified as persistent organic pollutants (POP) under the Stockholm Convention in 2001 due to inherent chemical stability and toxicity. However, accidental exposure to PCDD/F continues to occur due to their environmental persistence.

Recent revision of maximum allowable limits of PCDD/F in food items was recently lowered 0.02-3.5 pg/g wet weight within the European in 2021, placing greater emphasis on analysis sensitivity and selectivity. As soil is a key vector for contamination transport, accurate and reliable analysis of soil at trace level concentrations is of importance. Gas chromatography magnetic sector mass spectrometry has been the gold standard in the analysis of PCDD/F over the past few decades providing the required mass resolution (i.e., >10,000 resolving power at 10% valley) and accuracy (i.e., 5 pm) for global compliance, such as that laid out the U.S. Environmental Protection Agency (EPA) method 1613.2 However, greater demands are being placed on analytical instrumentation with higher mass resolution and system flexibility are needed to increase scope into unknown chemical exposures. Recent advances ion mass spectrometry technology can provide greater mass resolution with sub ppm mass accuracy, providing alternative tools for PCDD/F analysis that still fulfill strict compliance criteria in accredited methodology.

In this study, we evaluated the performance of a GC Orbitrap high mass resolution spectrometer operating at 60,000 mass resolution (200 m/z full width at half maximum (FWHM)) to deliver trace analysis of PCDDs / PCDFs at current maximum allowable limits in with soil according to criteria laid out in EPA method 1613.2

Materials and Methods:
Soil extraction: A two-gram soil sample was extracted in a bi-phasic mixture of acetonitrile/hexane (4 mL:4 mL) followed by centrifugation at 3,000 rpm. A 100 μL aliquot of the hexane layer was then evaporated to dryness, spiked with 30 μL of the lowest calibration standard, and reconstituted in 100 μL nonane.

For the soil analysis a GC-Orbitrap mass analyzer was utilized. A 1.5 μL sample volume was injected using a split/splitless injector with chromatographic separation performed on a TraceGold(TG)-Dioxin (60 m × 0.25 mm, 0.25 μm) capillary GC column using previously described condition.3 Prior to analysis, electron voltage energy was optimized together with ion source optics to provide optimal sensitivity using a 5 pg/μL standard of 2, 3 , 7 , 8 Tetrachlorodibenzodioxin (TCDD). Quantification of soil analysis was performed using isotopic dilution in Chromeleon Chromatography Data System software with evaluation of performance criteria (i.e., retention time tolerance, ion confirmation ratio, relative response factor deviation) as part of the Dioxin Analyzer workflow.

Results: The influence of electron energy on the analytical response to 2, 3, 7, 8-TCDD (5 pg/μL) can be seen in Figure 1A. Lowest response was observed at the standard 70 eV electron voltage while optimal sensitivity was found at a setting of 40 eV. Evaluation of optimized settings was evaluated using a soil extract (no sample clean-up) spiked at the low fg range with PCDD/F standard (45 fg on column) can be seen in Figure 2B, where a 2-3 times improvement in analyte response was observed between electron energy settings of 40 and 70 eV respectively.

![Figure 1. (A) Signal response of 5 pg·μL-1 2,3,7,8-TCDD calibration standard (extracted mass: m/z 321.8930) at electron energies from 30 – 70 eV and (B) area response (counts*sec) of 2,3,7,8-TCDD in spiked soil extract (45 fg on column) at 40 and 70 eV.](image-url)
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P-125  Meeting the challenges of Dioxin analysis and more with GC-Orbitrap high mass resolution capabilities

Figure 1. (A) Signal response of 5 pg•µL−1 2,3,7,8-TCDD calibration standard (extracted mass: m/z 321.8930) at electron energies from 30 – 70 eV and (B) area response (counts*sec) of 2,3,7,8-TCDD in spiked soil extract (45 fg on column) at 40 and 70 eV.

Linear calibration response was observed across all PCDD/F congeners spanning over three orders of magnitude (0.05 – 100 pg/µL) with response deviation being less than 6 %. Compliance with performance of soil analysis were within criteria specified under EPA 1613 for PCDD/F analysis within environmental. Quantification and confirmation ion retention time and confirmation ratio were within tolerance limits of ± 0.01 and ± 15 %, respectively. Average relative response factor was observed to deviate no more that 20% across all calibration points. Analysis run time (i.e., 45 min) was also significantly reduced using the TG-Dioxin column compared to conditions outlined in EPA 1613 using a DB-5 stationary phase without loss in chromatographic resolution efficiency.

High mass resolution is a required for the analysis of PCDD/F with a resolving power ≥10,000 at 10% valley or 5% peak height across all target masses according to EPA Method 1613. At a mass resolution setting of 60,000, this criterion was achieved all targeted analytes across the entire mass range. An example of this can be observed in Figure 2 for our heaviest target analyte, octachlorodibenzodioxin (OCDD).

Simulating the required mass resolution for OCDD under EPA 1613, a mass resolution of approximately 23,000 is needed (Figure 2a), which approaching the resolution limits of time of flight (TOF) mass spectrometers. At a mass resolution setting of 60,000 which was used in our analysis, a resolving power of approximately 40,000 was achieved (Figure 2b), well surpassing the criteria needed for EPA 1613 compliance.

Quantified results from the analysis of a soil extract (no clean-up) spiked with PCDD/F (congener concentration range: 30 – 3000 fg/µL) were in good agreement with spike concentrations (Figure 3). 1,2,3,4,6,7,8-HpCDD, OCDD, and OCDF were detected above spiking levels. Similar findings were also reported for the same sample analysis using GC MS/MS,3 confirming the presence of these congeners in the soil sample prior to spiking.
**Conclusion:** Our results demonstrate that GC-Orbitrap high mass resolution instrumentation can provide an alternative approach that provides sensitive and accurate trace analysis for PCDD/F within accepted performance criteria, while providing laboratories technological compacity to broaden their scope to investigate unknown and emerging chemical exposures.

**Acknowledgements:**
Please include acknowledgements and funding sources.

**References:**
**Introduction:** Food and environmental testing laboratories are under pressure to continuously deliver data that is compliant with regulations. However, the current crisis surrounding the helium gas supply is making laboratory operations unsustainable from both economic and throughput standpoints. Helium is the ideal carrier gas for gas chromatography-mass spectrometry (GC-MS) due to its inert nature and fast-pumping efficiency, making it the preferred option to maintain optimal performance of GC-MS instrumentation. Whereas reduction of the helium consumption is a logical first step, some laboratories face challenges to obtain any helium supply. Hydrogen in turn, is a more cost efficient alternative and can be even generated directly in the lab. However, switching to hydrogen as carrier gas often means a change in performance usually requires re-optimization of the method. In this poster, the use of hydrogen for the analysis of polyaromatic hydrocarbons (PAHs) and selected pesticides will be shown to illustrate how reliable and efficient analysis can nonetheless be obtained.

**Materials and Methods:** The performance of a single quadrupole GC-MS system, equipped with a novel SSL injector design allowing to decouple the pressurizing gas flow from the actual carrier gas flow was operated using hydrogen as a carrier gas for the analysis of PAHs according to U.S. EPA Method 8270E.2

The same injector design was used on a triple quadrupole GC-MS/MS system for the analysis of pesticides according to the DG SANTE guidelines.2 Two matrices (baby food and honey) were tested, and the results evaluated for a total of 181 pesticides. Key figures of merit were investigated for each method, including linearity, accuracy and limits of detection.

**Results:** For PAH analysis, a linear calibration model fit was used, and excellent correlation (coefficient of determination $R^2 > 0.99$) was obtained for all analytes, spanning a calibration range of more than three orders of magnitude between 2.0 to 5,000 pg $\mu$L$^{-1}$. Variation in the calibration response factors was well below 15%, thus, demonstrating the identical performance when using hydrogen in place of helium.

For pesticide analysis, more than 97% of the 181 evaluated compounds showed a linear response within the investigated concentration range in both the tested matrices, with 99% of the analytes showing RSD $\leq$10% at a level of 0.05 mg/kg.

**Discussion and Conclusion:** The proposed setup for both GC-MS as well as GC-MS/MS provides a viable alternative for laboratories performing the testing for critical contaminants and looking at ways to circumvent helium price increases or potential supply shortages. Whilst the use of hydrogen may have a negative impact on detection sensitivity for some compounds (and hence the ability to meet established detection limit requirements), the use of a sensitivity optimized ion source together with a few changes in the choice of transitions can offset these and allow to achieve comparable performance at reduced running costs.

The higher chromatographic efficiency of hydrogen versus helium allowed for an improved of the method speed in both cases. Using the same carrier flow and oven program conditions, an improvement of analysis speed in between 7-10 % could be realized for the analysis of PAH (equivalent to approximately one minute) while separation between isobaric compounds was maintained.

**References:**
2. Analytical Quality Control and Method Validation Procedures for Pesticide Residues Analysis In Food And Feed. SANTE 11312/2021
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P-127 A preliminary study for sensitive analysis of organic fluorine by combustion ion chromatography

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Introduction:
Per- and polyfluoroalkyl substances (PFAS) have received worldwide attention because of their environmental persistence and toxicity. PFAS have been widely used in consumer products for decades, and a number of PFAS have been found in environmental matrices. A robust analytical method is needed to manage a number of PFAS. Combustion ion chromatography (CIC) is useful for comprehensive analysis of PFAS, whereas the applications of CIC for low-concentration measurements have been limited due to two major issues[1]: co-elution of fluoride with organic acids, and high level of instrumental blank. Thus, the aims of this study are 1) to resolve fluoride from the interfering organic acids, and 2) to reduce fluoride contamination from the analytical procedure.

Materials and Methods:
The separation of fluoride from organic acids was examined with two types of columns: IonPac AS20 (2 mm i.d.×250 mm length, 7.5 μm, Thermo Fisher Scientific Inc.) and IonPac AS30 (2 mm i.d.×250 mm length, 5.5 μm, Thermo Fisher Scientific Inc.), which have different ion-exchange properties. IonPac AS20 (77.5 μeq/column) was used as the column with the highest ion-exchange capacity in a previous study[1], whereas IonPac AS30 (119 μeq/column) is recently developed as the column with the highest ion-exchange capacity. The combustion ion chromatography (CIC) used in this study consists of an automated combustion unit (AQF-2100H; Nittoseiko Analytech Inc.) and an ion chromatography system (Integrion RFIC; Thermo Fisher Scientific Inc.).

Results and Discussion:
Chromatograms of fluoride and organic acids obtained with IonPac AS20 and IonPac AS30 columns are shown in the Figure 1. Fluoride and organic acid peaks were fully resolved with IonPac AS30, which has a 1.5-fold greater ion-exchange capacity than IonPac AS20. In a previous study with IonPac AS17 (7.5 μeq/column) and IonPac AS20, fluoride and organic acid peaks were resolved with the higher capacity column (IonPac AS20). Our result shows a better separation of fluoride and organic acids than the previous study. We then conducted several experiments to check for the sources of CIC instrumental blanks. The initial background level of fluoride from the analytical procedure was 68 ng-F. Fluoride was detected after absorbing the combusted gases without injecting any sample, suggesting that the source of contamination was mainly present within the gas lines or the gases themselves. Gas tubes made up of PTFE were then replaced with either stainless steel or polyethylene tubing. Gases were also replaced to high-purity gases (Ar: 99.9999%, O2: 99.99995%). Following these modifications, the background levels of fluorine in instrumental blanks decreased by more than 50-fold, compared to the level found in the initial CIC. In this study, the limit of detection (LOD) of organofluorine was 1.2 ng-F, which is lower than that in a recent study (8.2 ng-F)[2]. Further modifications are needed to attain lower background level of CIC.

Figure 1: Chromatograms of fluoride and organic acids obtained with IonPac AS20 and IonPac AS30 columns.

Acknowledgments:
This study was supported by a Grand-in-Aid for Scientific Research (B) from the Japan Society for the Promotion of Science (No. JP21H03614) and the Environment Research and Technology Development Fund (3-2102: JPMERF20213002) of the Environmental Restoration and Conservation Agency of Japan.

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Abstract
Polycyclic aromatic hydrocarbons (PAHs) are compounds containing two or more fused aromatic hydrocarbon rings. They are formed during pyrolytic processes that involve incomplete combustion of organic matters, including forest fire, fuel burning, barbecuing and roasting. Foods may be contaminated by PAHs by environmental pollution and/or food processing steps. Benzo[a]pyrene (B[a]P) is considered one of the most toxic PAHs. It is classified as carcinogenic to humans (Group 1) by the International Agency for Research on Cancer (IARC).

In Asia, foods contaminated with B[a]P have been reported from time to time. In view of the carcinogenicity of B[a]P and safety concern of food for infant and children, a maximum permitted concentration of B[a]P in infant formulae and follow-up formulae was set at 1 µg/kg in Hong Kong under the Harmful Substances in Food (Amendment) Regulation 2021. A method developed for the determination of B[a]P in infant formulae and follow-up formulae by gas chromatography with tandem mass spectrometry will be presented.

In gist, commercially available infant formulae and follow-up formulae in the Hong Kong market were used in this study. A powdered sample of infant formula or follow-up formula was weighed and d12-B[a]P was added as the internal standard. Deionised water and 25% ammonia solution were added and the mixture was heated at 65 °C for 10 minutes. After cooling down to room temperature, ethanol was added and the mixture was transferred into a Mojonnier flask for the subsequent extraction of fat-soluble B[a]P with diethyl ether and petroleum ether. The organic layer was separated for solvent evaporation. The residue was then reconstituted with cyclohexane and loaded into a molecularly-imprinted polymer solid-phase extraction (MIP-SPE) column for purification. The eluate, dried and reconstituted with pentane, was passed through a deactivated silica gel column. d12-Perylene was added to the filtrate as the injection standard. B[a]P in the sample was analysed using GC-MS/MS (Thermo Scientific TSQ 8000 Evo) equipped with the DB-35ms column (30 m × 0.25 mm × 0.25 µm). The method was validated using infant formula and follow-up formula samples spiked with B[a]P at 0.025 – 0.50 µg/kg.

References
Introduction: Per- and Polyfluoroalkyl substances (PFAS) are used in a wide range of consumer products and industrial applications due to their excellent heat resistance and water repellency. There are reportedly thousands of compounds that fall under the category of PFAS, and their chemical characteristics vary depending on their carbon chain length and functional groups. While PFAS have useful features, they can pollute surface water, groundwater, soil, and air in various regions around the world, and have adverse effects on human health. This has led to a worldwide tightening of regulations on PFAS. In addition, a highly sensitive analysis is required because even trace amounts of PFAS affect human health. Among regulatory targets, PFAS analysis in aqueous matrices is the most regulated, and various analytical methods have been developed. ASTM D8421-22 is the test method for determination of PFAS in aqueous matrices by LC/MS/MS. In this method, short-chain to long-chain PFAS are specified as the compounds to be measured. ODS columns, commonly used in reversed-phase chromatography, have weak column retention of compounds with short carbon chains, making it difficult to obtain good peak shapes under some conditions. Dilution with water is the easiest way to improve the peak shapes, but it is not suitable for sensitive analysis. In this study, we evaluated the effect of column dimensions by the co-solvation and direct injection method with reference to ASTM D8421-22 and investigated an analytical method that enables highly sensitive analysis of a wide range of PFAS.

Materials and Methods: The analytical condition was investigated based on using Shim-pack GIST-HP C18, 50 mm x 2.1 mm I.D., 3 µm (Shimadzu Corporation) as the analytical column and Shim-pack GIST C18, 50 mm x 3.0 mm I.D., 5 µm (Shimadzu Corporation) as the delay column. The standard solutions for EPA draft method 1633 (Wellington Laboratories) were mainly used for calibration, and the column dimensions and gradient program were optimized. In order to analyze various PFAS simultaneously with high sensitivity, parameters related to ionization that could be set with the LCMS-8060NX (Shimadzu Corporation) were investigated. Using the developed method for ASTM D8421-22, analytes that co-solvated by a 1+1 ratio of sample and methanol were measured directly. We confirmed the repeatability of the peak areas of the standard solution and the recovery rate of the sample to which 160 ppt equivalent was added to the wastewater.

Results: When a column with a small particle size was used, the peak shape distorted. The reason is that the small particle size made the solvent effect worked strongly by suppressing the mixing of the sample solvent and the mobile phase. On the other hand, the longer the column length and the wider the inner diameter, the better the peak shape, probably because the symmetry of the column exit was improved by the diffusion. Under the optimized conditions, the half-width of the peak of PFPrA (C∼HF∼O∼), which had a short carbon chain and a poor peak shape, was improved to less than half its full width. In addition, short-chain to long-chain PFAS (C3-C14) could be measured sensitively with good peak shape. As a result of ionization conditions for simultaneous analysis of various PFAS, it was found that CID gas pressure, interface temperature and DL temperature had a significant effect on the sensitivity of each compound. Interestingly, the interface temperature has the opposite impact on the sensitivity depending on the functional group. For example, in higher interface temperature, some groups of PFAS gain the sensitivity but other groups lose the sensitivity. Using optimized method, good results were obtained with spike recoveries from 70 to 130 % for most compounds.

Discussion and Conclusion: Using a column of the Shim-pack series, we optimized the conditions for trace PFAS analysis of multiple components. Some of the parameters involved in ionization have a large effect on sensitivity. Therefore, when simultaneously analyzing PFAS compounds with different chemical characteristics with high sensitivity, it may be necessary to investigate the ionization conditions suitable for the PFAS compounds to be measured. Using the optimized method for ASTM D8421-22 with a column having favorable dimensions, good results can be obtained for short-chain to long-chain PFAS compounds. Also, the optimized method does not require any equipment modification, so it is possible to measure EPA draft method 1633 on the same equipment.

Progress in Methods for POPs Analysis

P-130  Quantification of linear and branched Perfluoroalkyl sulfonates and carboxylates (PFAS) in hay from a contaminated area in Germany

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Introduction: Due to the long-term industrial production of PFASs by electrochemical fluorination (EFC), not only linear isomers are generated, but also branched (br) isomers are formed as byproducts. In the case of perfluorooctanesulfonic acid (PFOS), approximately 70-80 % of the linear structure and 20-30 % of branched isomers are produced (Buck et al., 2011). Branched isomers have been found in a wide range of environmental and biological samples, e.g., in surface water, soil, chicken eggs, fish, and human blood (Schulz et al., 2020). Currently, there is no strong focus on the quantification of branched PFAS isomers in plant-based matrices used for feed. Hay is an important feed material for livestock and can contribute to the PFAS exposure to humans through the food chain. Different effects of branched and linear PFAS isomers have been observed in some health studies (Bao et al. 2017). For better understanding of possible health associations of branched PFASs to humans and animals, it is important to pay attention to isomers during measurement and quantification. A hay sample from a contaminated area in North Rhine-Westphalia, Germany, was used to develop a method for the quantification of linear and branched PFASs in hay. The distinctive PFAS profile of linear and branched isomers was determined, and the challenges of the quantification of branched species were highlighted.

Materials and Methods: 10 mL distilled water, 10 mL acetonitrile (ACN), and 150 µL formic acid were added to 0.3 g of the homogenized hay sample. The sample was then shaken for five minutes and placed in an ultrasonic bath for another five minutes. 4 g MgSO4 and 1 g NaCl were added and the sample was shaken for 1 min and centrifuged at 3500 rpm for 15 min. The ACN extract was then cleaned by dispersive solid-phase extraction (dSPE). The falcon was then shaken for five minutes and centrifuged at 3500 rpm for 15 minutes. The supernatant was removed and further cleaned with graphite carbon black. The measurement of ten replicates was carried out on an LC-MS/MS instrument.

Results: Several branched isomers of the sulfonates and carboxylates (C5 to C14) were detected in the hay sample. Perfluorononanesulfonic acid (br-PFNS), with a content of 117 µg/kg (88 % dry weight, dw), and Perfluorodecanesulfonic acid (br-PFDS), with a content of 86.5 µg/kg (88 % dw), are the dominant branched isomers. The ratio of the branched to linear form is 2.4 for PFNS and 0.9 for PFDS. Perfluorobutanoic acid (PFBA) has the highest content with 150 µg/kg (88% dw). Due to a lack of standards, the content varies depending on the transition used for quantification.

Table 1. Content in µg/kg related to 88% dry weight (n=1) of the four dominant br-PFAS depending on the transitions.

<table>
<thead>
<tr>
<th>PFAS analyte</th>
<th>m/z [a]</th>
<th>m/z [b]</th>
<th>µg/kg (88% dw) [a]</th>
<th>µg/kg (88% dw) [b]</th>
<th>average µg/kg (88% dw) [a/b]</th>
</tr>
</thead>
<tbody>
<tr>
<td>br-PFNS</td>
<td>549 → 80</td>
<td>549 → 99</td>
<td>114</td>
<td>377</td>
<td>261</td>
</tr>
<tr>
<td>br-PFDS</td>
<td>599 → 80</td>
<td>599 → 99</td>
<td>83</td>
<td>227</td>
<td>155</td>
</tr>
<tr>
<td>br-PFOS</td>
<td>499 → 80</td>
<td>499 → 99</td>
<td>11,6</td>
<td>6,04</td>
<td>8,82</td>
</tr>
<tr>
<td>br-PFOA</td>
<td>413 → 369</td>
<td>413 → 169</td>
<td>9,12</td>
<td>32,7</td>
<td>20,91</td>
</tr>
</tbody>
</table>

Discussion and Conclusion: The analyzed hay sample demonstrates the possible occurrence of br-PFAS up to perfluorotetradecanoic acid (br-PFTeDA) in the environment. The results depend strongly on the transition used for quantification. This shows that further harmonization on how to quantify the branched isomers without a standard is necessary.

References:
Progress in Methods for POPs Analysis

P-131  Fast determination of non-dioxin-like PCB in meat and fat of animal origin in contaminant control plan samples

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1. Introduction:
Early in 2023 the Contaminant Control Plan was established by the Delegated Regulation (EU) 2022/931 together with the Implementing Regulation (EU) 2022/932. Annex I No. 1 of Regulation (EU) 2022/931 specifies combinations of contaminants or groups of contaminants in various animal products. Food of animal origin should be analyzed for halogenated persistent organic pollutants (hPOPs) or metals. The group of hPOPs with maximum levels includes only the group of dioxins/PCB or PFAS. The minimum frequency of testing is specified in Regulation 2022/932 Annex I No. 1. A disproportionate number of dioxin and/or PCB analyses have to be carried out in animal foodstuffs. In order to be able to analyze these samples quickly and efficiently, a rapid method for non-dioxin-like PCB (ndl-PCB) has been developed that meets the criteria of Regulation 2017/644.

2. Materials and Methods:
As part of the national residue control plan, samples were obtained from slaughterhouses. Since the maximum levels apply to fat, no quantitative determination in fat was performed. The muscle meat or the adipose tissue was homogenized in a cutting mill with sodium sulfate. The homogenate was treated with n-pentane in an iodine flask and extracted in an ultrasonic bath for 15 minutes. The fat-containing solvent was filtered through a cleaned pleated filter and separated on a rotary evaporator. 13C-labelled ndl-PCB, purchased from Promochem, Germany, were added as internal standards to an aliquot of 0.2 g fat. A total volume of 2 ml was achieved by addition of cyclohexane.

The extract was injected into a Freestyle GPC/SPE system from LCTech Germany with a sulfuric acid SPE column (test column from LC-Tech, Germany, 6 ml; 5 g 44% (sulfuric acid on silica gel). Within 20 minutes, the ndl-PCB were eluted with 20 ml of cyclohexane. The extract was concentrated to 200 µl in an evaporation chamber and transferred to a 200 µl vial. The extract was dried under a gentle nitrogen stream, dissolved in 100 µl recovery standard and measured with a GC-HRMS system (DFS, Thermo Fisher Bremen) on an HT-8 column (50m x 0.22mm x 0.25µm) using a 3-point calibration curve.

3. Results:
The aim of this work was to develop a method, that complies to the criteria laid down in Regulation 2017/644 for the determination of ndl-PCB. The recovery of the internal standards has to be between 60 and 120% and the minimum limit of quantification has to be 2 ng/g fat for the individual congeners. Only small amounts of fat can be weighed in due to the small bed volume of the sulfuric acid SPE. In general, the sample weight was in the range of 0.1 to 0.2 g and the final volume was 100 µl. With this method it has been possible to achieve limits of quantification for the individual congeners of less than 0.2 ng/g fat. This is a factor of 10 lower than the target of the method. The gas chromatographic separation of non-dioxin-like PCB from interferences, in particular from co-eluting PCB, was achieved with the HT-8 GC column.

The performance criteria of Regulation 2017/644 could be achieved not only at the maximum level, but also in the lower ng/g range. The intermediate laboratory precision (RSDR) for PCB 138, PCB 153 and PCB 180 at levels of 2 ng/g fat is 1 to 3%. The trueness is also less than 5% when tested with proficiency test material. The limit of quantification for the lower-chlorinated ndl-PCB (PCB 28, 52 and 101), which are only present at very low concentrations in food of animal origin, is 0.2 ng/g fat. Based on these limits, a minimum limit of quantification of 1.2 ng/g fat for the sum of ndl-PCB can be achieved with the method described here.

4. Discussion:
It can be concluded that a minimum limit of quantification of 0.2 ng/g fat is sufficient to detect the presence of ndl-PCB when comparing the performance criteria of the described method with the occurrence and levels of ndl-PCB found in food and feed in Europe (1). For the determination of ndl-PCB, the method is therefore sufficiently sensitive, specific and robust.

5. References
(1) Barbara Gallani, Anna Boix, Alessandro Di Domenico, Roberto Fanelli: Occurrence of NDL-PCB in food and feed in Europe Organohalogen Compounds Vol. 66 (2004) 3561- 3569
Progress in Methods for POPs Analysis

P-132 Automation of Solid Phase Extraction (SPE)-Clean-up for the Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Food

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Introduction:
Since 01 January 2023 maximum levels for 4 PFAS (PFOS, PFOA, PFNA, PFHxS) and their sum in certain food matrices are established for official food control in the EU according to Commission Regulation (EU) 2023/915 [1]. Additionally maximum limits of quantification (LOQ) are set for monitoring of PFAS in certain matrices [2]. With this new EU regulation and recommendation there is an increased need to expand capacities to analyze samples, preferably with the same or less manpower. Furthermore, the Guidance Document [3] specifies validation parameters that methods of monitoring or compliance testing in the EU should achieve. Automation of the clean-up has the potential to reduce workhours invested in comparison to manual methods, while achieving the necessary LOQ needed to check the new maximum levels for PFAS.

Materials and Methods:
The extraction and clean-up method are based on the recommended method in section 6.4.3.1. of the Annex of the Guidance Document [3]. The primary matrix used was beef. The samples were spiked with internal standard as well as three concentrations of a PFAS-standard mixture including 41 PFAS. After extraction of the samples with acetonitrile in an ultrasonic bath, the extracted solutions were cleaned up with an automated SPE-System with two columns: first an activated carbon column followed by a polymeric weak anion exchange column. The resulting solutions were blown to dryness with nitrogen and the residue resolved in 500 µL of a mixture of formic acid (1%) and MeOH (1:2). Seven calibration solutions in a range from 0.01 µg/kg to 10 µg/kg were prepared. Chromatographic separation and measurement were conducted with a well-established LC-MS/MS setup using an ESI-source and working in MRM mode. Separation column: C18 Reversed Phase. Trapping column C18 Reversed Phase. Solvents: A) 2 mM NH₄Acq and B) 998 mL MeOH + 2 mL 1 M NH₄Acq. Starting pressure 380 Bar. Injection volume 10 µL.

Results:
The Guidance Document recommends validating methods for a range starting at or below the maximum level of quantification for monitoring of 0.1 µg/kg in beef [2] and at the least including the maximum levels at the upper end [3]. According to this the spiked levels were chosen to be 0.1 µg/kg, 0.5 µg/kg and 1 µg/kg respectively of each of the 41 PFAS analyzed. Most of the 41 substances reliably showed apparent recoveries between 80% and 120% as well as recoveries of the internal standards between 60% and 120% for all spiked levels. Most important, all 4 PFAS regulated in the EU showed these apparent recoveries for spiked concentrations between 0.1 µg/kg and 1 µg/kg. The relative standard deviation for the 4 regulated PFAS was between 4 % and 11 % for all spiked concentrations.

Discussion and conclusion:
The range of spike levels fits the conditions set by the Guidance Document for methods of monitoring and compliance testing [3]. Taking into account the sufficient recoveries and the determined precision, acceptable LOQ are achievable. An automated clean-up of PFAS could possibly be more efficient than a manual method, but this depends highly on the regular sample throughput. Due to the possibility to run the clean-up system unattended, laboratory staff could have more time for other tasks.

Acknowledgements:
We would like to thank the European Commission for the financial support of the work of the European Union Reference Laboratory for Halogenated POPs in Feed and Food, Freiburg, Germany.

References:
2. Commission Recommendation (EU) 2022/1431 of 24 August 2022
Introduction: Hazardous and Noxious Substances (HNS) encompass a wide range of materials, including hazardous liquid substances, liquefied gases, and bulk or packaged solid dangerous goods. These substances are primarily generated during ship transportation or released into the water system as a result of industrial processes at offshore facilities, ultimately entering the marine environment. Due to the persistence of certain HNS in seawater and marine sediments, they pose substantial risks to both human health and marine life. Therefore, it is of utmost importance to develop accurate analytical methods for the detection of these HNS in seawater and marine sediments, enabling efficient monitoring of their long-term presence. The main aim of this study was to enhance the analysis of such HNS in marine sediments through optimization techniques.

Among the various substances present in HNS, this study focused on the analysis of 11 volatile organic compounds (VOCs). In previous research, a NaCl supersaturated solution, known for its salting-out effect, was commonly employed as the solvent to enhance extraction efficiency. However, in this study, we investigated the use of room temperature ionic liquids (RTILs) as alternative extraction solvents, considering their growing application across different fields. By comparing the recovery rates of the target compounds between RTILs and NaCl supersaturated solution, we aimed to determine the optimal solvent for our analysis.

Materials and Methods: For this study, sediment samples were obtained from Bijin Island in Tongyeong, South Korea, which is recognized as a pristine location. In order to extract the target compounds, three hydrophilic RTILs and a NaCl supersaturated solution were employed as extraction solvents. The selection of 11 specific target compounds, including chloroform, benzene, trichloroethylene, bromodichloromethane, toluene, tetrachloroethylene, dibromochloromethane, m,p-xylene, and o-xylene, was based on the identification of these compounds in sediment samples from previous studies.

For quantitative and qualitative analysis, calibration was carried out using standards containing the target compounds and internal standards. The analysis was performed using headspace-GC/MS.

Results: The results of this study revealed that volatile organic compounds were not detected in the sediment samples collected from Bijin Island. To investigate the detection capabilities of different extraction solvents, the sediment samples were spiked with same amounts of standards and extracted using NaCl supersaturated solutions, as well as three different room temperature ionic liquids (RTIL-1, RTIL-2, and RTIL-3). Further analysis involved comparing the recovery rates of the target compounds using the different solvents. It was observed that the sample extracted with RTIL-1 (1-ethyl-3-methylimidazolium ethyl sulfate, [EMIM][ESO4]) exhibited the most favorable recovery rates among the four solvents tested. Consequently, RTIL-1 was selected as the extraction solvent for subsequent analyses. Using RTIL-1, target compounds were detected with Method Detection Limits (MDLs) ranging from 0.06 to 0.11 ng/g and Limits of Quantification (LOQs) ranging from 0.2 to 0.36 ng/g. The precision of the analysis, expressed as the relative standard deviation, ranged from 5% to 7%, while the accuracy, calculated as the percentage recovery, exceeded 100% for all compounds. This suggests that the extraction and analytical methods employed in the study were effective in recovering the target compounds from the sediment samples.

Discussion and Conclusion: In this study, the application of RTILs as a new solvent for the analysis of VOCs in marine sediments was investigated. To the best of our knowledge, hydrophilic RTILs have not been previously used for VOC analysis in marine sediments. Based on these findings, future research aims to further explore and compare the efficiency of RTILs and NaCl supersaturated solutions for in situ sample analysis of contaminated marine sediments near coastal industrial facilities. This investigation will provide valuable insights into the suitability and effectiveness of RTILs as a solvent for HS-GC/MS analysis, potentially improving the assessment and monitoring of VOC contamination in marine environments.

Acknowledgments: This research was supported by Korea Institute of Marine Science & Technology Promotion (KIMST) funded by the Ministry of Oceans and Fisheries, Korea (20210660, 'Development of Technology for Impact Assessment and Management of HNS discharged from Marine Industrial Facilities').
**Progress in Methods for POPs Analysis**

P-134  Semi-Automated Fast Extractable Petroleum Hydrocarbons Fractionation

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**Introduction:** Soil contamination from gasoline, diesel fuel, heating oil, kerosene, jet fuel leaks or spills is a common occurrence and a global environmental concern. In the United States, environmental testing labs identify fuel using the EPA Total Petroleum Hydrocarbon (TPH) method 8015B. The semi-volatile fraction is identified by the distribution pattern displayed when analyzed via GC-FID. Petroleum products are composed of over 250 compounds, making the analysis of all of them difficult. Some individual states have created separate methods for extractable petroleum hydrocarbons (EPH) and volatile petroleum hydrocarbons (VPH). These EPH methods take a more toxicological approach and evaluate the composition of aliphatic and aromatic compounds in an extracted sample.

A low-cost, 6-position, parallel semi-automated cleanup system was developed for fast and reliable extraction of aliphatic and aromatic compounds from complex extracts. The extracts are fractionated using neutral silica gel and the aliphatic and aromatic fractions are analyzed separately using GC-FID, giving a more accurate assessment of health risks.

**Material and Methods:** The semi-automated system uses a rotary workstation and a 6-channel parallel pump to perform the entire sample extraction in two stages. The system is run with a multi-pump that has Start, Reset, and Flow rate switches (5 or 10 mL/min), and individual Enable switches for each of the six channels. It also has electronic readouts for the back pressure present on each of the six channels. If one of the channels gets over pressurized an alarm goes off. It uses one pre-packaged 6 g neutral silica column to do the fractionation. This is an important feature because the use of certified columns packed in a clean room greatly reduces the risk of cross-contamination from the laboratory background.

In the first stage the column is conditioned with dichloromethane and hexane (both 30 mLs) to waste. In the second stage the sample is diluted in 9 mLs hexane (optional), spiked with surrogates and loaded onto the silica. The column is eluted with 10 mL hexane, collecting the aliphatic fraction. It is then eluted with 35 mL dichloromethane, collecting the aromatic fraction. The fractions are reduced to 1 mL under a nitrogen stream at 30 oC. The analysis is done with GC-FID or GC/MS. Total processing time is 20-25 min. Up to six samples can be run in parallel.

**Results:** Linear aliphatics (C9-C36) were analyzed with recoveries between 77-96%. Seventeen aromatics (PAHs) were analyzed with recoveries between 97-111%. RSDs were < 9%. The semi-automated EPH system with certified 6 g silica gel columns gives excellent and fast separation of aliphatic hydrocarbons from aromatic hydrocarbons (PAHs). The combination of the EPH system and silica columns demonstrates consistent and reproducible data with a reliable high throughput. Several concentrations run (2.5, 12.5 and 25 ug/mL) gave very good Method Detection Level data that showed values between 0.5-4.0 ug/mL.

Laboratories doing EPH analysis often do not measure individual compounds but instead measure the entire peak area as a total sum parameter over a certain retention time range. A study we did with this technique produced very good results and showed that the semi-automated system produced as good total sums of compounds eluting as when the EPH extraction was carried out manually.

**Conclusions:** The semi-automated system for EPH fractionation gives very good results with great recoveries and fraction separation (aliphatics vs aromatics). Standard deviations are low and the whole procedure is fast, carried out typically within 25 min. Method Detection Limits are good enough to make the system usable by commercial environmental laboratories.
Polychlorinated dibenzo-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are a group of chemically related compounds that are toxic and persistent organic pollutants (POPs). These compounds are restricted internationally under the Stockholm Convention and due to the bioaccumulative nature of these compounds, it is essential to monitor them at ultra trace levels in food and environmental samples. Traditionally these compounds have been analyzed using magnetic sectors with electron ionization sources which require expert users to obtain consistent results. As there is a growing concern for the analysis of these compounds, more user-friendly technology is essential to analyze potentially contaminated samples. Depending on the regulation the usage of GC MS/MS systems are prohibited or the congener patterns in order to identify the source of possible contamination is needed, the GC Quadrupole Time of Flight offers a novel alternative.

Materials and Methods:
Residue grade n-hexane, dichloromethane, and toluene were purchased from J.T. Baker (Phillipsburg, NJ, USA). Standard solutions for 2,3,7,8-PCDD/Fs specified by EPA Method 1613, including those for EPA1613 CVS, LCS, ISS were supplied by Wellington Laboratories Inc (Ontario, Canada).

The analyses were performed on an Agilent 8890B GC system combined with an Agilent 7250 Quadrupole Time of Flight system equipped with an electron impact ionization (EI) source and operated in full scan mode, acquiring the Data in full Acquisition using 2 Hz. A 60m × 250 μm × 0.25 μm VF-Xms column was used with Helium as the carrier gas, the column flow rate was kept at 1 mL/min. A 1 µL sample was injected in hot splitless mode. The total GC run time was 60 min.

Results
The Agilent 7250 facilitates the screening and quantitation of polychlorinated dioxins, furans, and PCBs at low levels in difficult matrix samples and provides results with high certainty. (see an EPA 1613 CS1 analysis in Figure 1).

Discussion and Conclusion:
The Agilent 7250 GC QTOF facilitates the screening and quantitation of polychlorinated dioxins, furans, and PCBs at low levels in difficult matrix samples and provides results with high certainty. The proposed HRMS measurement scheme uses profile full acquisition data is a valuable solution for screening for dioxins/furans and dl-PCBs at the relevant MRL levels.
Progress in Methods for POPs Analysis

P-136  Analysis of PCDD/F in Food and Feed by Intuvo 9000/7010 GC-QQQ System

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² Agilent Technologies, 2850 Centerville Rd, 19808 Wilmington, DE, United States

1 Introduction
Dioxins and furans are persistent environmental pollutants that have been extensively studied and shown to bioaccumulate in the environment. Historically, high resolution mass spectrometry (HRMS) was needed to confirm and quantify trace levels of dioxins, as in EPA Method 1613B. However, as of June 2014, the European Union (EU) has instituted regulation (709/2014) governing the levels of PCDDs, PCDFs, dioxin-like PCBs, and non-dioxin-like (NDL) PCBs in food and feed that enables the use of gas chromatography/tandem quad mass spectrometry (GC/TQ MS) systems in confirmatory testing for compliance with EU MLs. This change was due to the realization that triple quadrupole mass spectrometers could provide performance similar to that seen with HRMS systems and was previously demonstrated on an Agilent 7890B/7000C GC/TQ MS system.1 With the introduction of the Intuvo 9000 GC and its direct heating technology, new possibilities for a faster separation can be explored for this critical analysis.

2 Materials and Methods

Experimental
The evaluation of performance was demonstrated using an Agilent Intuvo 9000 GC configured with a split/splitless inlet, coupled to an Agilent 7010 Series triple quadrupole using the high efficiency ion source (HES). Two methods were developed to meet various needs. The first method has a run time of 52.5 minutes and uses a temperature program that may also be applied to traditional GC ovens. A faster method was developed, taking advantage of the direct column heating available on the Intuvo 9000 GC. The instrument conditions for both methods are listed in Table 1.

Sample preparation
The most frequently used methods for the determination of PCDD/PCDF and DLPCB in foodstuffs and animal feed combine fat extraction (Soxhlet) with cleanup steps using different column chromatographies (silica gel coated with sulphuric acid, florisil, alumina, and active carbon). Manual dioxin sample preparation is tedious and comprehensive; multicolumn automated systems have been made to automate dioxin sample extraction to reduce analysis times and attempt to reduce costs according to the 1613 method.

Table 1. Intuvo 9000 GC System parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>52.5 Minute Method</th>
<th>31.12 Minute Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>GC Conditions</td>
<td>p/n 5181-3315 and 5190-2293</td>
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</tr>
<tr>
<td>Injection Port Liner</td>
<td>1 µL</td>
<td>1 and 0.5 µL</td>
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<tr>
<td>Pulsed Splitless</td>
<td>60 psi for 0.6 minutes; 50 mL/min at 0.8 minutes</td>
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<tr>
<td>Column</td>
<td>DB-5MS UI (60 m × 250 µm, 0.25 µm)</td>
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<td>Oven</td>
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<td>20 °C/min to 220 °C (15 minutes)</td>
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<td>5 °C/min to 330 °C (2 minutes)</td>
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<td>330 °C</td>
<td>330 °C</td>
</tr>
<tr>
<td>Source Temperature</td>
<td>280 °C</td>
<td>280 °C</td>
</tr>
<tr>
<td>Quadrupole Temperature</td>
<td>150 °C</td>
<td>150 °C</td>
</tr>
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### Table 2. Compounds tested (LCS = Labeled Compound Standard ISS = Internal Syringe Standard)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>2378-TCDD</td>
<td>Native</td>
</tr>
<tr>
<td>2378-TCDF</td>
<td>Native</td>
</tr>
<tr>
<td>12378-PCDD</td>
<td>Native</td>
</tr>
<tr>
<td>12378-PCDF</td>
<td>Native</td>
</tr>
<tr>
<td>23478-PCDF</td>
<td>Native</td>
</tr>
<tr>
<td>123478-HxCDD</td>
<td>Native</td>
</tr>
<tr>
<td>123478-HxCDF</td>
<td>Native</td>
</tr>
<tr>
<td>123678-HxCDD</td>
<td>Native</td>
</tr>
<tr>
<td>123789-HxCDD</td>
<td>Native</td>
</tr>
<tr>
<td>123789-HxCDF</td>
<td>Native</td>
</tr>
<tr>
<td>234678-HxCDF</td>
<td>Native</td>
</tr>
<tr>
<td>1234678-HpCDD</td>
<td>Native</td>
</tr>
<tr>
<td>1234678-HpCDF</td>
<td>Native</td>
</tr>
<tr>
<td>1234789-HpCDF</td>
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<tr>
<td>OCDF</td>
<td>Native</td>
</tr>
<tr>
<td>1234-TCDD-ISS</td>
<td>Syringe Standard</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Compound</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>2378-TCDD-LCS*</td>
<td>ISTD</td>
</tr>
<tr>
<td>2378-TCDF-LCS*</td>
<td>ISTD</td>
</tr>
<tr>
<td>12378-PCDD-LCS*</td>
<td>ISTD</td>
</tr>
<tr>
<td>12378-PCDF-LCS*</td>
<td>ISTD</td>
</tr>
<tr>
<td>23478-PCDF-LCS*</td>
<td>ISTD</td>
</tr>
<tr>
<td>123478-HxCDD-LCS*</td>
<td>ISTD</td>
</tr>
<tr>
<td>123478-HxCDF-LCS*</td>
<td>ISTD</td>
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<tr>
<td>123678-HxCDD-LCS*</td>
<td>ISTD</td>
</tr>
<tr>
<td>123678-HxCDF-LCS*</td>
<td>ISTD</td>
</tr>
<tr>
<td>123789-HxCDD-ISS</td>
<td>Syringe Standard</td>
</tr>
<tr>
<td>123789-HxCDF-LCS*</td>
<td>ISTD</td>
</tr>
<tr>
<td>234678-HxCDF-LCS*</td>
<td>ISTD</td>
</tr>
<tr>
<td>1234678-HpCDD-LCS*</td>
<td>ISTD</td>
</tr>
<tr>
<td>1234678-HpCDF-LCS*</td>
<td>ISTD</td>
</tr>
<tr>
<td>1234789-HpCDF-LCS*</td>
<td>ISTD</td>
</tr>
<tr>
<td>OCDD-LCS*</td>
<td>ISTD</td>
</tr>
<tr>
<td>OCDF-LCS*</td>
<td>ISTD</td>
</tr>
</tbody>
</table>
**POSTERS**

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### Table 3. Calibration curve for the 52.5-minute method; 1 µL injection volume.

<table>
<thead>
<tr>
<th>fg/µL</th>
<th>Tetra</th>
<th>Penta</th>
<th>Hexa</th>
<th>Hepta</th>
<th>Octa</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>50</td>
<td>100</td>
<td>100</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>L2</td>
<td>200</td>
<td>400</td>
<td>400</td>
<td>800</td>
<td>800</td>
</tr>
<tr>
<td>L3</td>
<td>1000</td>
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<td>L4</td>
<td>4000</td>
<td>8000</td>
<td>8000</td>
<td>16000</td>
<td>16000</td>
</tr>
<tr>
<td>L5</td>
<td>20000</td>
<td>40000</td>
<td>40000</td>
<td>80000</td>
<td>80000</td>
</tr>
<tr>
<td>L6</td>
<td>80000</td>
<td>160000</td>
<td>16000</td>
<td>320000</td>
<td>320000</td>
</tr>
</tbody>
</table>

### Table 4. Compound specific coefficient of determination results for the 52.5-minute method

<table>
<thead>
<tr>
<th>Analyte</th>
<th>R2</th>
</tr>
</thead>
<tbody>
<tr>
<td>2378-TCDD</td>
<td>0.99997</td>
</tr>
<tr>
<td>12378-PCDD</td>
<td>0.99983</td>
</tr>
<tr>
<td>123478-HxCDD</td>
<td>0.99978</td>
</tr>
<tr>
<td>123678-HxCDD</td>
<td>0.99998</td>
</tr>
<tr>
<td>123789-HxCDD</td>
<td>0.99928</td>
</tr>
<tr>
<td>1234678-HpCDD</td>
<td>0.99998</td>
</tr>
<tr>
<td>OCDD</td>
<td>0.99999</td>
</tr>
<tr>
<td>2378-TCDF</td>
<td>0.99991</td>
</tr>
<tr>
<td>12378-PCDF</td>
<td>0.99987</td>
</tr>
</tbody>
</table>

### Results

A standard containing Native PCDDs, Native PCDFs, syringe standards, and 13Clabeled internal standards (Table 2) was evaluated with both the 52.5-minute and 31.12-minute method. Chromatograms displaying the separations under both sets of conditions are shown in Figures 1 and 2. Calibration curves were analyzed for regression analysis against both separation methods. For the original, longer method, a six-point calibration curve was analyzed, with concentration details provided in Table 3. Correlation of determination was used as an evaluation of the linearity, and the resulting values are shown in Table 4. A more detailed statistical evaluation of the data is provided in Table 5. In this table, the transitions used for each target compound is provided, along with instrument-specific limits and signal-to-noise values. The instrumental limit of quantitation (iLOQ) was calculated using 10 replicate injections at the lowest calibration point.
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Method 2: Faster temperature program
Using the accelerated temperature program shown in Table 1, a second calibration curve was generated using a 1 µL injection of prepared standards shown in Table 6. In similar fashion to the longer method evaluation, the calibration standards were run in sequence, then processed using a data system where correlations of determination were generated for each compound. The transitions applied to each compound were kept consistent with those provided in Table 5. Table 7 displays the correlation values.

Table 5. RT, MRM transitions, LOQ, and LOD

<table>
<thead>
<tr>
<th>Name</th>
<th>RT</th>
<th>Transition</th>
<th>Conc. RSD</th>
<th>MDL (fg/µL)</th>
<th>LOQ</th>
<th>LOD</th>
<th>Noise</th>
<th>S/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>2378-TCDF</td>
<td>26.64</td>
<td>303.9 → 240.9</td>
<td>2.6</td>
<td>3.8184</td>
<td>13.5334</td>
<td>4.06</td>
<td>1.92</td>
<td>18.54</td>
</tr>
<tr>
<td>2378-TCDD</td>
<td>27.835</td>
<td>319.9 → 256.9</td>
<td>3.9</td>
<td>5.7684</td>
<td>20.445</td>
<td>6.1335</td>
<td>1.61</td>
<td>14.62</td>
</tr>
<tr>
<td>12378-PCDF</td>
<td>33.638</td>
<td>339.8 → 277.0</td>
<td>4.9</td>
<td>14.8674</td>
<td>52.6945</td>
<td>15.8083</td>
<td>2.02</td>
<td>17.6</td>
</tr>
<tr>
<td>23478-PCDF</td>
<td>35.359</td>
<td>339.8 → 277.0</td>
<td>6.4</td>
<td>20.1087</td>
<td>71.271</td>
<td>21.3813</td>
<td>1.99</td>
<td>21.43</td>
</tr>
<tr>
<td>12378-PCDD</td>
<td>35.921</td>
<td>355.9 → 292.9</td>
<td>6.5</td>
<td>20.0992</td>
<td>71.2375</td>
<td>21.3713</td>
<td>1.48</td>
<td>19.69</td>
</tr>
<tr>
<td>123478-HxCDF</td>
<td>39.96</td>
<td>373.8 → 310.9</td>
<td>6.4</td>
<td>19.4782</td>
<td>69.0366</td>
<td>20.711</td>
<td>1.55</td>
<td>24.45</td>
</tr>
<tr>
<td>123678-HxCDF</td>
<td>40.141</td>
<td>373.8 → 310.9</td>
<td>4.6</td>
<td>14.2778</td>
<td>50.6047</td>
<td>15.1814</td>
<td>1.55</td>
<td>25.08</td>
</tr>
<tr>
<td>123478-HxCDF</td>
<td>41.148</td>
<td>373.8 → 310.9</td>
<td>3.4</td>
<td>10.0481</td>
<td>35.6132</td>
<td>10.684</td>
<td>1.96</td>
<td>22.2</td>
</tr>
<tr>
<td>123478-HxCDD</td>
<td>41.237</td>
<td>389.8 → 326.9</td>
<td>4.2</td>
<td>12.5373</td>
<td>44.4357</td>
<td>13.3307</td>
<td>1.51</td>
<td>23.82</td>
</tr>
<tr>
<td>123678-HxCDD</td>
<td>41.533</td>
<td>389.8 → 326.9</td>
<td>8.1</td>
<td>24.7315</td>
<td>87.6558</td>
<td>26.2947</td>
<td>1.45</td>
<td>25.78</td>
</tr>
<tr>
<td>123789-HxCDD</td>
<td>41.737</td>
<td>389.8 → 326.9</td>
<td>7.3</td>
<td>21.5291</td>
<td>76.3055</td>
<td>22.8916</td>
<td>1.55</td>
<td>26.1</td>
</tr>
<tr>
<td>123789-HxCDF</td>
<td>42.135</td>
<td>373.8 → 310.9</td>
<td>4.9</td>
<td>14.8626</td>
<td>52.6774</td>
<td>15.8032</td>
<td>1.39</td>
<td>30.68</td>
</tr>
<tr>
<td>1234678-HpCDF</td>
<td>44.133</td>
<td>407.8 → 344.8</td>
<td>4.0</td>
<td>23.4349</td>
<td>83.06</td>
<td>24.918</td>
<td>1.76</td>
<td>46.85</td>
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<tr>
<td>1234678-HpCDD</td>
<td>45.674</td>
<td>423.8 → 360.8</td>
<td>3.5</td>
<td>20.7641</td>
<td>73.594</td>
<td>22.0782</td>
<td>1.9</td>
<td>34.19</td>
</tr>
<tr>
<td>1234789-HpCDF</td>
<td>46.105</td>
<td>407.8 → 344.8</td>
<td>8.7</td>
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<td>187.7979</td>
<td>56.3394</td>
<td>1.52</td>
<td>52.61</td>
</tr>
<tr>
<td>OCDD</td>
<td>48.83</td>
<td>457.7 → 394.8</td>
<td>2.8</td>
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<td>59.2874</td>
<td>17.7862</td>
<td>1.33</td>
<td>44.22</td>
</tr>
<tr>
<td>OCDF</td>
<td>48.995</td>
<td>441.7 → 378.8</td>
<td>2.5</td>
<td>14.9213</td>
<td>52.8855</td>
<td>15.8656</td>
<td>1.35</td>
<td>49.63</td>
</tr>
</tbody>
</table>
Table 6. Calibration curve for the 32.12-minute method; 1 µL injection volume

<table>
<thead>
<tr>
<th>fg/ul</th>
<th>Tetra</th>
<th>Penta</th>
<th>Hexa</th>
<th>Hepta</th>
<th>Octa</th>
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</thead>
<tbody>
<tr>
<td>SC1</td>
<td>20</td>
<td>40</td>
<td>40</td>
<td>80</td>
<td>80</td>
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<tr>
<td>SC2</td>
<td>80</td>
<td>40</td>
<td>160</td>
<td>320</td>
<td>320</td>
</tr>
<tr>
<td>SC3</td>
<td>400</td>
<td>200</td>
<td>800</td>
<td>1600</td>
<td>1600</td>
</tr>
<tr>
<td>SC4</td>
<td>1600</td>
<td>800</td>
<td>3200</td>
<td>6400</td>
<td>6400</td>
</tr>
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<td>SC5</td>
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<tr>
<td>SC6</td>
<td>32000</td>
<td>16000</td>
<td>64000</td>
<td>128000</td>
<td>128000</td>
</tr>
</tbody>
</table>

Table 7. Correlations of determination for the accelerated method.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>R2</th>
</tr>
</thead>
<tbody>
<tr>
<td>2378-TCDD</td>
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</tr>
<tr>
<td>12378-PCDD</td>
<td>0.99984</td>
</tr>
<tr>
<td>123478-HxCDD</td>
<td>0.99975</td>
</tr>
<tr>
<td>123789-HxCDD</td>
<td>0.9999</td>
</tr>
<tr>
<td>123678-HxCDD</td>
<td>0.99981</td>
</tr>
<tr>
<td>1234678-HpCDD</td>
<td>0.99998</td>
</tr>
<tr>
<td>OCDD</td>
<td>0.99999</td>
</tr>
<tr>
<td>2378-TCDF</td>
<td>0.99992</td>
</tr>
<tr>
<td>12378-PCDF</td>
<td>0.99985</td>
</tr>
<tr>
<td>23478-PCDF</td>
<td>0.99991</td>
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<tr>
<td>123478-HxCDF</td>
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<td>123678-HxCDF</td>
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<td>1234678-HpCDF</td>
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<tr>
<td>1234789-HpCDF</td>
<td>0.99999</td>
</tr>
<tr>
<td>OCDF</td>
<td>0.99993</td>
</tr>
</tbody>
</table>

Advances in mass spectrometry, most notably the HES available on the 7010 TQ, shows promise for lower detection, and subsequently smaller sample volumes. To evaluate the impact of the HES on the accelerated separation method, a smaller injection volume was added, providing data for both 1 and 0.5 µL sample injections at the lowest calibration point. Table 8 shows the comparison of injection volume, using RSD% for seven replicates of the SC1 standard listed in Table 6.

Conclusions:
Regulatory agencies are recognizing the ability of tandem quadrupole systems to effectively identify and quantify concerning dioxin and furan compounds, as shown by European Union Commission Regulations No. 2017/644 and No. 709/2014, which adds GC/TQ as an option for confirmatory analysis of certain foodstuffs. Compared to high resolution MS systems (HRAM), GC/TQ is a more affordable system to analyze potentially contaminated samples. Advances in MS sources, most notably the HES option evaluated in this work, allow for improved detection with smaller injection volumes, without a loss in data confidence or precision. Likewise, advances in gas chromatography, such as the Intuvo 9000 system, accelerate separations with direct heating capabilities, which opens a pathway for rapid screening and faster throughput. In this work, two separation methods were developed to demonstrate performance using faster separations and smaller sample volumes. These outcomes are possible due to the partnership between the innovative technology embedded in the Intuvo 9000 GC and the robust performance of the 7010 TQ Mass spectrometer.

7 References
Riener, J., 2016, Validation of a Confirmatory GC/MS/MS Method for Dioxins and Dioxin-like PCBs to Meet the Requirements of EU Regulation 709/2014. Agilent Technologies Application Note, publication number 5991-6590EN
Progress in Methods for POPs Analysis

P-137  Field-deployable LC-MS platform for on-site screening of per-, and poly-fluoroalkyl substances (PFAS) in environmental samples

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¹ Trajan Scientific and Medical, 7 Argent Place, Ringwood, VIC 3134, Australia
² ADE Consulting Group, Williamstown North, VIC 3016, Australia

Introduction:
Per- and poly-fluoroalkyl substances (PFAS) are ubiquitous in the environment. Their ingress into the global food chain has led to measurable levels in nearly the entire human population in developed countries, with real and potential health effects reported worldwide. There are a few screening methods that offer the capability to detect total PFAS, but none of them provide the required selectivity and sensitivity to measure individual PFAS at concentrations as low as ng/L for environmental water samples, or sub-µg/kg for soils. Liquid chromatography coupled with triple quadrupole mass spectrometry (LC-QqQ-MS) is the most effective laboratory-based technique for determination of PFAS in various types of samples [1]. However, sample transport to the central laboratory has created significant latency in sample analysis. With minimal sample preparation on site, a proof-of-concept field-deployable LC-single quadrupole-MS (LC-Q-MS) platform was demonstrated for addressing on-site environmental monitoring requirements [2]. Results achieved in an enhanced field screening workflow based on the proof-of-concept platform have been compared with those obtained from a LC-QqQ-MS method performed in a centralized laboratory.

Materials and Methods:
A portable briefcase-sized capillary LC system (Trajan, VIC, Australia) coupled with a compact single quadrupole MS was configured for in-field analysis. Contaminated soil samples were weighed into polypropylene tubes and extracted in a methanol-ammonia solution with addition of surrogate. Following centrifugation, the supernatants were syringe filtered (0.22µm). Internal standard was added to each sample prior to analysis. For LC-Q-MS analysis, filtrates were concentrated under nitrogen and then reconstituted for injection. For LC-QqQ-MS analysis in the NATA-accredited laboratory, an aliquot was split and diluted for injection. Data acquisition was performed in negative ion electrospray and selective ion monitoring (SIM) mode, respectively.

Results:
The field-deployable LC-Q-MS platform was deployed for on-site testing of soil samples collected from contaminated sites. Method sensitivity for six PFAS compounds ranged from 1.4-5.2 ng/g, with wide dynamic range (20-12,500 ng/g) and excellent linearity ($R^2 > 0.997$). On-site test indicated that three of six PFAS compounds were identified in some of the collected soil samples, with the calculated concentrations ranging from 10.51 ng/g (PFOS) to 15.96 ng/g (PFHxA). The highest concentration PFHxS detected in soil samples was measured at 13.18 ng/g. On-site processing and analysis of 40 samples was demonstrated in an 8-hour time frame with two full-time staff. Results of the determined PFAS in the processed samples obtained from the LC-Q-MS system have shown high correlations with those obtained from the laboratory-based LC-QqQ-MS system.

Discussion and Conclusion:
A small footprint, field-deployable LC-Q-MS platform has demonstrated higher flexibility and sustainability as compared to a LC-QqQ-MS system, which is usually impractical for frequent transportation and deployment in the same setting. Despite lower detection sensitivity, results achieved from the LC-Q-MS system in such field screening workflow along with minimal sample preparation procedures have demonstrated high correlations with those from the centralized laboratory. Furthermore, improvements on sample processing and analysis throughputs of such workflow have been demonstrated as compared to the previous proof-of-concept study. Capabilities of the demonstrated field screening workflow and utility requirements for on-site analysis are to be verified in different site scenarios.

References:
Passive air sampling plays an important role in global monitoring of persistent organic pollutants (POPs) and other semi-volatile organic compounds (SVOCs), from small-scale and short-term local studies up to long-term air monitoring programmes (Gioia et al., 2006; Schuster et al., 2010). Because they need no power source and little maintenance, passive air samplers (PAS) like polyurethane foam (PUF) disks, sorbent-impregnated polyurethane foam (SIP) disks, and semipermeable membrane devices filled with triolein (SPMDs), provide a cost-effective way of collecting long-term and time-integrated data on air pollution, fulfilling obligations of international chemicals regulations such as the Stockholm Convention (UNEP, 2001) and the UN/ECE Protocol on POPs (UN/ECE Convention on Long-Range Transboundary Air Pollution, 1998).

To use passive air samplers for quantitative measurement of air concentrations, the sampling rate, R, or the PAS-air partition coefficient, $K_{\text{PAS-Air}}$, has to be known. A range of calibration data and partitioning coefficients have been reported, for example by Ockenden et al. (2001; (1998), Li and Wania (2021), and the research group led by Tom Harner at Environment and Climate Change Canada (e.g. Abdollahi et al. (2017); Harner et al. (2013); Herkert et al. (2018); Parnis et al. (2016); Saini et al. (2019)). However, field sampling conditions such as temperature and wind speed can be difficult to replicate and maintain over extended periods and detailed data are not always available in, for example, remote areas. Therefore, performance reference compounds (PRCs) are often used to calibrate the sampling rate in situ.

PRCs are analytically non-interfering organic compounds that cannot be found in the environment (Moeckel et al., 2009; Soderstrom and Bergqvist, 2004). They are added to the sampler before its deployment and the amount lost will depend on their physico-chemical properties, exposure time, and wind speed. Suggestions regarding the ideal mass loss of PRC range between 20 – 80% (Gioia et al., 2006; Moeckel et al., 2009; Soderstrom and Bergqvist, 2004), and >60% (Pozo et al., 2006). Conventionally, a mixture of $^{13}$C-labelled PCBs has been used as PRCs for the sampling of PCBs in passive samplers, due to their physico-chemical properties being nearly identical to those of their respective native compound. However, this has a number of drawbacks. For example, these labelled compounds might already be integrated in the analytical method. They are also not 100% chemically pure (a very small amount in the standard will be the respective unlabelled compound), which could increase the method limit of quantification to a value higher than the amount sampled from the air. Furthermore, when detection methods other than mass spectrometry are used, such as an electron capture detector (ECD), isotope-labelled standards cannot be used because they cannot be differentiated from the native target compound. Finally, for some methods with low method recoveries, the use of the isotope-labelled standards most similar to the target compounds may be necessary to guarantee the quality of the results, and suitable replacements may be difficult to find.

In this study, we have tested the use of monofluorinated PCBs (F-PCBs) as alternative performance reference compounds for the sampling of PCBs. Monofluorinated PCBs have one hydrogen atom in their molecule replaced by one fluorine atom. Like isotope-labelled PCBs, F-PCBs have similar physico-chemical properties to their corresponding PCBs, with F-containing aromatic compounds showing similar characteristics in many aspects to those of the corresponding non-fluorinated parent compounds. (Luthe et al., 2009; Sott et al., 2008). Polyurethane foam passive air samplers (PUF-PAS), spiked with (a) $^{13}$C-labelled PCBs or (b) F-PCBs as PRCs and (c) without PRCs were deployed for up to half a year, and subsequently their loss of PRCs and uptake of PCBs were determined. The results of this study show possible advantages and downsides for the use of F-PCBs compared to $^{13}$C-labelled PCBs as performance reference compounds.

2. Materials and methods:
Polyurethane foams (PUF) disks (thickness 13.5 mm, outside diameter 140 mm) were obtained from Klaus Ziemer GmbH, Germany. Acetone (HPLC grade) and hexane (HPLC grade) for sampler pre-extraction and sample extraction and clean-up were bought from Fisher Scientific, UK. Disks were spiked with a $^{13}$C-PCB mixture (CIL standards, USA) or, alternatively, with a “Dutch Seven F-PCB” mixture from Chiron UK Ltd. Details about the F-PCB standard can be found in Table 1. Activated silica/acid silica columns for clean-up were prepared using silica gel 60 (0.060-0.2 mm, 70-230 mesh, Alfa Aesar, UK), sulfuric acid (96% extra pure, Acros Organics, UK), and sodium sulfate anhydrous (0.63-2.0 mm coarse granules, Merck KGaA, Germany). Gel permeation chromatography columns were each filled with 8 g Bio-Beads S-X3 from Bio-Rad Laboratories, UK. PUF disks were rinsed with deionised water (Purite Ltd, UK) and dried in a drying oven at 30 °C for a week. The dry disks were then pre-extracted using a soxhlet apparatus for 2 x 24 hours with a hexane/acetone 1:1 mixture and subsequently dried in a desiccator. The dry disks were spiked with either 5 ng of $^{13}$C-PCB mixture, 5 ng of F-PCB mixture, or not spiked at all. All disks were stored in solvent-rinsed amber glass jars in a freezer for clean samples at temperatures below -20 °C until deployment. Sampling was carried out between the 5th of September 2019 and the 4th of March 2020 (181 days) at the Hazelrigg Field Station near Lancaster University, UK. In order to protect the PUF samplers from adverse weather conditions, they were deployed in labelled stainless steel assemblies described by Melymuk et al. (2021), which were assembled on site. Of each sampler type,
(with 13C-PCBs, with F-PCBs, without PRCs) eight samplers were deployed, two of which would be collected after 2, 4, 13, and 26 weeks, respectively. Additionally, two samplers of each type were prepared but not deployed, to become '0 weeks' samples. One laboratory blank each was analysed for all sampler types. After collection, the PUF disks were spiked with 625 pg recovery standard and soxhlet-extracted with 300 ml hexane for 16 hours. Sample clean-up consisted of a silica (6 g) – acid silica (6 g; silica:sulfuric acid 2:1) column and a gel permeation chromatography column (GPC, 8g). The final extracts were analysed using a Thermo DSQ II GC-MS system, equipped with an Agilent CP-Sil 8 CB GC column (50m x 0.25mm x 0.12µm).

Table 1. F-PCBs used in the study with their respective homologue group and IUPAC Number.

<table>
<thead>
<tr>
<th>Hom. group</th>
<th>IUPAC No.</th>
<th>Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trichloro-</td>
<td>3'-F-CB-28</td>
<td>3'-Fluoro-2,4,4’trichlorobiphenyl</td>
</tr>
<tr>
<td>Tetrachloro-</td>
<td>3-F-CB-52</td>
<td>3-Fluoro-2,2',5,5'-tetrachlorobiphenyl</td>
</tr>
<tr>
<td>Pentachloro-</td>
<td>3'-F-CB-101</td>
<td>3'-Fluoro-2,2',4,5,5'-pentachlorobiphenyl</td>
</tr>
<tr>
<td></td>
<td>5'-F-CB-118</td>
<td>5'-Fluoro-2,3',4,4',5-pentachlorobiphenyl</td>
</tr>
<tr>
<td>Hexachloro-</td>
<td>5'-F-CB-156</td>
<td>5'-Fluoro-2,3,3',4,4',5-hexachlorobiphenyl</td>
</tr>
<tr>
<td></td>
<td>3'-F-CB-166</td>
<td>3'-Fluoro-2,3,4,4',5,6-hexachlorobiphenyl</td>
</tr>
<tr>
<td>Heptachloro-</td>
<td>5'-F-CB-190</td>
<td>5'-Fluoro-2,3,3',4,4',5,6-heptachlorobiphenyl</td>
</tr>
</tbody>
</table>

3. Results and discussion:

Performance Reference Compounds. Figure 1 shows the remaining amount of performance reference compounds (PRCs) (13C-PCBs or F-PCBs) in the PUF disks after their respective deployment period. The values are the average of the duplicate samples, and given in pg/sampler. In summary, F-PCBs showed a similar but slightly elevated depuration behaviour compared to 13C-PCBs of the same homologue group. Assuming an exact amount of 5000 pg of each compound had been added to the sampler before deployment, after six months 48% of F-PCB 28 was lost, compared to 36% 13C-PCB 28. F-PCB 52 loss was even higher with 53% after six months, and twice as high as for 13C-PCB 52 (25%). However, this compound showed slightly reduced concentrations even in the F-PCB spiked laboratory blank and the t=0 weeks / field blank sample, so this very low value may reflect an analytical problem in this study rather than its environmental behaviour. Of F-PCB 101 and 13C-PCB 101, 29% and 17% were lost, respectively, but only 11% of F-PCB 118, which also belongs to the penta-chlorinated group. Fluorinated hexa-CBs showed very little depuration (6% for F-PCB 156, 2% for F-PCB 166), which, due to analytical measurement uncertainties, may well mean that almost no fluorinated hexa-CBs were lost within the six-month deployment period at all. Likewise, for the isotope-labelled hexa-CBs 13C-PCB 138 and 13C-PCB 153, the amounts measured on the samplers at the end of the maximum deployment period were actually higher than the 5000 pg originally added. A relatively constant loss between 15% and 29% for F-PCB 190 and between 14% and 18% for 13C-PCB 180 would also seem to be method-related rather than genuine volatilisation, given that this apparent loss can already be observed after a deployment period of only two weeks. With a logKoa of 10.7 (Shoeib and Harner, 2002), PCB 180, like other hepta-CBs, would be unlikely to show significant volatilisation and therefore a reduction after only a few weeks of deployment.

Figure 1. Amount in pg/sampler of the performance reference compounds 13C-PCBs (left) and F-PCBs (right) remaining on the sampler at the start of the study (0 weeks) and after a deployment period of 2, 4, 8, 13, and 26 weeks.
Uptake of native PCBs. In order to investigate the possible impact of different PRCs on the sampling of native target PCBs, a range of PCBs were analysed in all samples. The results, in pg per sampler (average of two identical samplers), can be seen in Figure 2. Plots on the left-hand side show the sums of all congeners within a homologue group measured, while plots on the right-hand side show those congeners within the homologue group that are the native equivalents of the \( ^{13}\text{C}-\text{PCBs} \) added.

Generally, PCB levels and uptake rates were very similar for all three types of samplers (with F-PCBs, with \( ^{13}\text{C}-\text{PCBs} \), without PRCs added), as expected, possibly with slightly higher amounts of tetra-, penta-, and hexa-PCBs found in the samplers without PRCs. In addition to somewhat higher depuration rates at the beginning of the study, probably due to higher temperatures, small increases in PCB levels during the first four weeks could be observed, which can be attributed to higher PCB air concentrations as a result of their increased volatilisation from primary and secondary sources with increasing temperatures. Hepta-CB concentrations including PCB 180 were only slightly above their limits of quantification (LOQs) in the first weeks and their concentrations should be viewed with this in mind.

One notable issue observed in this study was the presence of an impurity that co-eluted with PCB 101 in all samplers spiked with F-PCBs, including the associated laboratory blank and field blank, and also in the F-PCB spiking solution itself. It has not yet been determined if this impurity is PCB 101 itself - possibly a \(~4\%\) impurity of F-PCB 101 - or if it is a different compound showing the same behaviour as PCB 101 during the GC/MS analysis. This impurity amounts to ca. 200 pg/sampler, which is around 10% of the overall PCB 101 sampled after six months in this study. For comparable sampling sites with medium-
to-high PCB concentrations in the air, this can easily be compensated for by subtracting the laboratory blank value from all samples. However, as high blanks will also increase the limit of quantification (LOQ) calculated for each compound, for remote sampling sites with very low air pollution levels this could mean that the PCB 101 concentrations measured fall below the LOQ and it would therefore not be possible to report data for this congener. In any case, it would currently be advisable for every laboratory planning to use F-PCBs as part of their PCB method to run tests beforehand to rule out possible problems.

4. Conclusions:
F-PCBs have been demonstrated to work as PRCs, providing depuration rate constants similar to 13C-PCBs, as expected. F-PCBs can be used instead of 13C-PCBs to allow the utilisation of the latter as recovery or internal standards, or additionally, to increase the number of PRCs within the desired range of depuration, in order to obtain a more reliable and precise representation of the sampling conditions. Further detailed and robust studies to determine exact $K_{PAS,air}$ depending on meteorological conditions and sampler parameters will be helpful as an addition to currently existing datasets for a wide range of SVOCs.

However, before F-PCBs are integrated into any sampling or analytical method, possible impacts need to be assessed beforehand by each analytical group. In this study, only one batch of F-PCBs was used, so it is not clear if and to which extend the PCB-101 impurity is present in every standard solution purchased.

5. Acknowledgments:
The authors would like to thank Aimee Mariga for all her help in the lab and in the field.

6. References:
P-139  Monofluorinated PCBs (F-PCBs), a New Group of Potential Performance Reference Compounds for Passive Air Samplers


1 Introduction

Brominated flame retardants (BFRs) are used in a wide variety of commercial and industrial products, to retard or prevent the possible ignition of fire. The oldest and most widely used BFRs are polybromodiphenyl ethers (PBDEs) and hexabromocyclododecanes (HBCDs) [1]. Concerns have been raised regarding both BFR classes' potential dioxin-like toxicological properties and their endocrine-disrupting effects [2-4]. PBDEs and HBCDs were therefore both incorporated in the Stockholm Convention elimination list [5-8]. As a result of these bans, the use of other flame retardants, classified as “emerging” BFRs (eBFRs), is increasing. For example, decabromodiphenyl ethane (DBDPE), 1,2-bis(2,4,6-tribromophenoxy) ethane (BTBPE) and 2-ethylhexyl-2,3,4,5-tetrabromobenzene (EHTBB) are used in replacement of deca-, octa- and penta-BDE respectively. Due to their increased use, traces of these compounds have been detected in environmental matrices. Other new BFRs recently detected in the environment and in biota are pentabromoethyl benzene (PBEb), hexachlorocyclopentadienyl-dibromocyclooctane (HCDBCO), hexabromobenzene (HBB) and pentabromotoluene (PBT) [9]. It has been proven that many eBFRs have POPs properties, therefore they raise potential human health concerns [10]. The European Union issued Recommendation EU/118/2014 requesting Member States to monitor different BFRs classes in food, including PBDEs, HBCDs and eBFRs, to assess background contamination levels and estimate human exposure through diet [11]. In April 2022, the European Reference Laboratory (EURL) for halogenated POPs in Feed and Food published a Guidance Document on the Determination of Organobromine Contaminants setting analytical methods performances in order to allow harmonized monitoring of those contaminants in food [12]. However, no maximum limits for BFRs in food were yet defined. Several quantitative methods for PBDEs and HBCDs in food have been reported [13]. Some of these already included emerging BFRs [14]. The aim of the presented study was to set up and validate an efficient analytical method dealing with PBDEs, HBCDs and eBFRs, characterized by low solvent consumption and easily reproducible sample preparation. A QuEChERS like extraction was combined with a cleanup achieved on a Si-SPE/acidified Extrelut NT3 tandem columns assembly followed by size exclusion chromatography (GPC). The analysis was conducted by GC-MS/MS for PBDEs/eBFRs and LC-MS/MS for HBCDs. The proposed method ensures analytical accuracy and the low LOQs required by Recommendation 2014/118/EU for most of the analytes.

2 Materials and Methods

2.1 Sample preparation and analysis

Nine PBDE congeners (28, 47, 49, 99, 100, 153, 154, 183, 209), 3 HBCD isomers (a, b and g) and 9 eBFRs (pTBX, PBBz, PBT, PEBB, HBBz, EHTBB, HCDBCO, BTBPE and DBDPE) were analyzed in food samples (milk and muscle) (Table 1). The method was developed in isotopic dilution including a single sample preparation followed by a dual detection in GC-MS/MS (PBDEs and eBFRs) and LC-MS/MS (HBCDs). Twenty grams of sample were spiked with label internal standards (IS) and subjected to QuEChERS-like extraction (5 mL of ultrapure water, 15 mL ethyl Acetate, 3 g of sodium chloride and 6 g of anhydrous magnesium sulphate). After shaking and centrifuging, 10 mL of the upper organic layer were collected and concentrated under nitrogen stream. The residue was purified on an H2SO4 acidified Extrelut NT3/SPE Si 1 g/6 mL tandem columns assembly (eluting with 13 mL of hexane and only silica with further 10 mL of hexane/dichloromethane (2:1, v/v) and then by gel permeation chromatography (Gilson, Madison, Wisconsin, U.S.). The collected GPC sample was divided into two fractions and reduced to dryness. PBDEs/eBFRs analysis were performed in GC-MS/MS on a 7890A GC – 7000B triple-quadrupole mass analyser (Agilent Technologies, Palo Alto, California, U.S.). The collected GPC sample was divided into two fractions and reduced to dryness. PBDEs/eBFRs analysis were performed in GC-MS/MS on a 7890A GC – 7000B triple-quadrupole mass analyser (Agilent Technologies, Palo Alto, California, U.S.). The collected GPC sample was divided into two fractions and reduced to dryness. PBDEs/eBFRs analysis were performed in GC-MS/MS on a 7890A GC – 7000B triple-quadrupole mass analyser (Agilent Technologies, Palo Alto, California, U.S.), in solvent vent mode, on a DB-SHT column (15 m x 250 µm x 0.10 µm – Agilent Technologies). The HBCDs fraction was injected in LC-MS/MS on an ACQUITY I-Class Ultra Performance Liquid Chromatography / Xevo TQ-S micro IVD system (Waters, Milford, Massachusetts, U.S.), using a Kinetex XB-C18 column (2.6 µm 100Å, 100 x 2.10 mm - Phenomenex) with methanol (A) and ammonium acetate 2 mM in water solution (B) as mobile phases (ESI-).
Progress in Methods for POPs Analysis

P-140  PBDEs, HBCDs and emerging (eBFRs) brominated flame retardants simultaneous analysis in food with single sample preparation and dual detection

Table 1. Analytes and respective label internal and syringe standards

<table>
<thead>
<tr>
<th>N</th>
<th>Name</th>
<th>Acronym</th>
<th>Labelled Compounds (ISs)</th>
<th>Syringe Standards (SSs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Polybrominated diphenyl ethers (PBDEs)</td>
<td></td>
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<tr>
<td></td>
<td>2,4,4′-Tribromodiphenyl ether</td>
<td>BDE-28</td>
<td>[13C12] BDE-28</td>
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<td>2</td>
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<td>Decabromodiphenyl ether</td>
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<table>
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<th>Name</th>
<th>Acronym</th>
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<th>Syringe Standards (SSs)</th>
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<td></td>
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<td>pTBX</td>
<td>[13C12] PBBz</td>
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<td>Hexabromobenzene</td>
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<td>2-ethylhexyl-2,3,4,5-tetram bromobenzanoate</td>
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<td>[13C12-15C13-d12] EHTBB</td>
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<th>Name</th>
<th>Acronym</th>
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<th>Syringe Standards (SSs)</th>
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<td>a-HBCD</td>
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<td>2</td>
<td>1,2,5,6,9,10-Hexabromocyclododecanes</td>
<td>b-HBCD</td>
<td>[13C12] g-HBCD</td>
<td>[13C12] b-HBCD</td>
</tr>
<tr>
<td>3</td>
<td>g-HBCD</td>
<td>g-HBCD</td>
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<td></td>
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</tbody>
</table>

2.2 Quality Assurance/Quality Control

Background contamination was carefully monitored at any stage of the analytical process as previously described, and a strict quality control was implemented in each batch [13]. Regular participation to inter-calibration exercises organized by the EURL ensured external quality assurance.

2.3 Method validation

The initially developed PBDEs/HBCDs method was fully validated in all food categories, as already described [13]. The suitability of the same protocol also for the simultaneous analysis of eBFRs in milk and muscle tissue had to be demonstrated. Instrumental eBFRs linearity was studied by injecting three calibration curves, in 3 different days, at different concentration levels: 0.4, 1.0, 2.0, 4.0, and 10 ng/mL (IS: 4 ng/mL) for BTBPE; 1.0, 2.0, 5.0, 10, 20 and 50 ng/mL (IS: 20 ng/mL) for DBDPE; 0.1, 0.2, 0.5, 1.0, 2.0 and 5.0 ng/mL (IS: 2 ng/mL) for all the other analytes. Replicate analysis of uncontaminated chicken muscle and bovine milk were performed at two spiking level, in inter-laboratory reproducibility conditions (Table 2). Precision (repeatability and intra-lab reproducibility) was estimated at each level by ANOVA. Trueness was measured in terms of corrected recoveries (isotopic dilution) and labelled internal standards recoveries. Limits of quantification (LOQs) were estimated by replicated analysis on a blank matrix spiked at the lowest level for each analyte, assessing the repeatability/reproducibility and verifying the compliance with Horwitz theoretical values.
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Table 2. Validation plan

<table>
<thead>
<tr>
<th>Application field</th>
<th>Matrix</th>
<th>IS (µg/kg)</th>
<th>N</th>
<th>IS (µg/kg)</th>
<th>N</th>
<th>IS (µg/kg)</th>
<th>N</th>
<th>MMSt</th>
<th>BS</th>
<th>PB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle and tissues</td>
<td>Chicken muscle</td>
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<td>0.05</td>
<td>15</td>
<td>0.020</td>
<td>0.10</td>
<td>14</td>
<td>0.10</td>
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<tr>
<td></td>
<td></td>
<td>0.050</td>
<td></td>
<td>15</td>
<td>0.10</td>
<td></td>
<td>13</td>
<td>0.50</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Milk and dairy products</td>
<td>Cow milk</td>
<td>0.010</td>
<td>0.05</td>
<td>14</td>
<td>0.020</td>
<td>0.10</td>
<td>13</td>
<td>0.10</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>0.050</td>
<td></td>
<td>14</td>
<td>0.10</td>
<td></td>
<td>14</td>
<td>0.50</td>
<td>14</td>
<td></td>
</tr>
</tbody>
</table>

MMSt: matrix match standards - BS: blank samples – PB: procedural blanks

3. Results:
The PBDEs/HBCDs analytical method validation results were already presented [13]. New experiments were implemented to demonstrate its applicability to eBFRs analysis. Good linear responses were observed in the chosen linearity range for all the eBFRs ($R^2 > 0.997$). Validation experiments were performed on blank samples of chicken muscle and bovine milk spiked at two different concentration levels (Table 2). The validation study results for eBFRs, together with those previously obtained for PBDEs and HBCDs at the LOQ level (taken as the more critical level), are shown in Table 3. Apparent eBFRs recoveries (R%) were generally between 86-119%. R% < 85% were obtained for PBT and PBEB, for which the corresponding label IS was not available and the internal standardization was achieved using PBBz-13C6, the compound with the closer retention time. Labelled IS R% were in the range 70-104%. Lower recoveries were obtained only for EHTBB-d7-13C6 (32-66%). The RSD (relative standard deviation in repeatability conditions) and RSD R (relative standard deviation in intra-lab reproducibility conditions) were lower than or equal to 15% and 16%, respectively.

4. Discussion:
The sample preparation procedure was developed starting from the PBDEs/HBCDs method [13], making it applicable to eBFRs analysis, and tweaks made to be able to analyse simultaneously all the three BFR classes. Eight labelled PBDEs congeners (28-, 47-, 99-, 100-, 153-, 154-, 183- and 209-13C12), two HBCD isomers (α- and γ-HBCD-13C12) and five eBFR (PBBz-13C6, HBBz-13C6, EHTBB-d7-13C6, BTBPE-13C6 and DBDPE-13C6) were added at the beginning of the procedure, with the aim to minimizing both the loss of the analyte due to analytical process and the matrix effect. BDE-49, without the corresponding labelled analogues, was quantified with BDE-47-13C12, characterized by similar degree of bromination. b-HBCD was quantified using γ-HBCD-13C12. For pTBX, PBT and PBEB the label compound with the closer retention time was used (PBBz-13C6), while for HCDBCO the IS HBBz-13C6 was the choice. The syringe standards (BDE-77- and -138-13C12 and b-HBCD-13C12) were also introduced to correct for inter-injection fluctuations and to assess the labelled congener’s recoveries (Table 1). Method LOQs were set at the lowest analytes concentration tested in spiked blank samples giving a RSDR compliant with the Horwitz theoretical value: 0.005 mg/kg for PBDEs (209: 0.10 mg/kg), 0.010 mg/kg for HBCDs and eBFRs (BTBPE: 0.020 mg/kg; DBDPE: 0.10 mg/kg). Intra-laboratory reproducibility and calibration curve contributions were taken into account in the estimation of the method relative extended uncertainty (U%).

The validation data were compliant to the EURL Guidance Document on the Determination of Organobromine Contaminants, for most of the analytes: trueness between - 30 % to + 30 %, within-laboratory reproducibility < 20 %, LOQ 0.010 µg/kg w.w. (except for BDE-209, BTBPE and DBDPE), IS recovery between 30 – 140 % (Table 3). BFRs background laboratory contamination control is a big issue in each analytical batch in terms of extra cleaning procedures and incremented quality control. An accurate procedure to control the background contamination was implemented and control charts were populated [13]. Method accuracy for BFRs analysis was also checked participating to proficiency tests (PTs) organized by the EURL for Dioxins and PCBs. Different PTs were dispatched for PBDEs and HBCDs since 2018. In 2021 was distributed the first PT (Cod liver oil) including also eBFRs. The z-scores obtained applying the here presented method were all satisfactory.

5. Conclusions:
A single sample preparation and double detection (GC-MS/MS and LC-MS/MS) method for the analysis of three important classes of brominated flame retardants was developed and validated in muscle and milk. It enables the simultaneous determination of 9 PBDE congeners, 3 HBCDs and 9 eBFRs at very low level (LOQ between 0.005-0.100 mg/kg) using a QuEChERS-like extraction followed by two steps clean-up. Multiclass methods like the one described in the present paper may encourage BFRs monitoring in official control laboratories and enabling the collection of data aiming to better assess the low background food contamination levels and finally bring to maximum limit issuing.
6. Acknowledgments:
The authors gratefully acknowledge financial support from the Italian Health Ministry: RF-2019-12370587 MicroPLASTICs in edible aquatic organisms: ecotoxicological effects, transfer of chemical and biological CONtaminants and susceptibility to bacteria biodegradation (PLASTICON).

Table 3. Validation results: limit of quantification (LOQ), spiking levels, number of replicates (n), relative standard deviations in repeatability (RSDr) and intra-lab reproducibility conditions (RSDR), relative expanded uncertainty (Urel), apparent recoveries (Rapp) and surrogate labelled standards recoveries (ISR ± SD). Concentrations > LOQ and < 0.100 µg kg⁻¹, concentrations ≥ 0.100 µg kg⁻¹.

7. References:
Progress in Methods for POPs Analysis

P-140  PBDEs, HBCDs and emerging (eBFRs) brominated flame retardants simultaneous analysis in food with single sample preparation and dual detection


10. EFSA, 2012. Scientific Opinion on Emerging and Novel Brominated Flame Retardants (BFRs) in Food


Polycyclic Aromatic Hydrocarbons (PAHs) are a large group of organic compounds with two or more fused aromatic rings and are known to be carcinogenic. Human beings are exposed to PAHs mostly by intake of food. As these compounds are highly soluble in lipophilic matrices, edible oils can be an important source of contamination by PAHs. In 2011, EU Commission Regulation No 835/2011, amending Regulation 1881/2006, set maximum levels in edible oils to 2µg/Kg of benzo[a]pyrene individually, and 10 µg/Kg of benzo[a]pyrene, benzo[b]fluoranthene, chrysene and benzo[a]anthracene combined. Some matrices remain challenging to analyze. To preserve the integrity of analytical devices and to reach a certain limit of detection, it is often necessary to perform sample preparation. The molecularly Imprinted Polymer (MIP) technology applied to Solid Phase Extraction (SPE) brings higher selectivity in comparison to classic polymeric or silica based SPE. It allows a thorough sample cleaning prior to analysis.

For PAH analysis, both canola and olive oils were tested. To do so, they were spiked with a mixture of 8 PAHs at 2µg/kg which is compliant with the maximum levels fixed by European regulation. A sample preparation using Solid Phase Extraction (MIP) was carried out prior to GC-MSMS analysis. In a second set of experiments, PAHs analysis were performed on a "full spectrum" type CBD oil which is a more complex type of oil to analyze. The cleaning procedure was performed on the oil using a combination of a "Pass-through" SPE cleanup and a MIP cartridge. The analyses were carried out by GC-MS/MS and LC-FLD.

Recovery yields ranging from 83% to 95% were obtained in edible oils, (with relative standard deviation < 8%) and from 85% to 103% (with relative standard deviation <7%) from CBD oil.

Very good results were obtained with an efficient cleanup and satisfying recovery yields for all the matrices tested. Moreover, the sample cleaning was also demonstrated to be suitable for LC-FLD analysis. In the future, other matrices such as sediments should be tested.

GC-MS/MS analysis were carried out by the national reference laboratory LABERCA, Nantes (France)
Perfluorinated compounds (PFAS or PFC) are a family of molecules composed of fluorocarbon chains of variable length and a functional group such as carboxylic or sulfonic acid, for example. They have been widely used since the 1950s in many products, such as in fire-fighting foams, for non-stick and hydrophobic coatings, or as surfactants. Their composition makes them particularly chemically resistant, and they therefore tend to accumulate in organisms and in the environment. Perfluorinated compounds have come under increased scrutiny in recent years because of their suspected adverse effects on human health.

To reach the low limits of PFAS quantitation, it is often necessary to concentrate the samples prior to analysis. Solid Phase Extraction (SPE) based on polymers has proven to be effective for PFAS concentration and quantitation. Moreover, PFAS analysis may be tricky because they tend to be found everywhere (they can be progressively released from HPLC parts and tubing) and it may be hard for a laboratory to obtain clear blanks.

A list of 10 PFAS were tested in tap water. 500 mL of tap water was spiked with 10 PFAS at 24ng/L and then passed through a polymeric Solid Phase extraction cartridge to concentrate the PFAS prior to LC-MSMS analysis. For this study, the SPE procedure was both tested manually and with an automaton. During the analysis, a delay column was used to avoid contamination of PFAS coming from HPLC device.

Recovery yields ranging from 88% to 102% were obtained in tap water. The mean reproducibility of the method (relative standard deviation obtained over eight samples) was 4.3% (with a maximum of 12% for PFOS). These results are a combination of the results obtained by SPE processed manually and those carried out with an automaton.

Very good results were obtained in tap water with good recovery yields and reproducibility. Moreover, the use of an HPLC delay column permitted to avoid PFAS contamination from the LC device and solvents to reach low limits of detection. In the future, other matrices such as tissues should be tested.
Introduction: Sample preparation for conducting dioxin analysis in solids and semi-solids can be very complicated throughout the sample preparation workflow. Accelerated solvent extraction (U.S. EPA Method 3545A) was developed to meet the new requirements for reducing solvent usage in the preparation of solid, soil and sediment samples. The Thermo Scientific™ Extreva™ ASE™ Accelerated Solvent Extractor system is a newly developed system based on many proprietary technologies including gas assisted solvent delivery and parallel accelerated solvent extraction. This fully automated system combines extraction and evaporation capabilities into one instrument.

Materials and Methods: This work will demonstrate the advancements of an analytical method to determine dioxins using a fully automated solvent extraction and in-line concentration system, the Thermo Scientific™ Extreva™ ASE™ Accelerated Solvent Extractor. This system utilizes gas assisted extraction that results in a more efficient and controlled extraction. Gas assisted extraction can be more effective as compared to static extraction methods. In addition, when done in parallel, this new technique yields many advantages. Furthermore, the solvent evaporation process is fully incorporated into the automated workflow ensuring sample integrity within the closed system for the determination of 17 PCDDs and PCDFs.

Results: Two batches of experiments for dioxins in soil were conducted each batch had four samples. One was conducted with 170mL of extract solvent and the other set of samples were extracted with 85mL of extraction solvent. All samples showed acceptable internal standard recoveries for dioxins under the conditions tested. This testing protocol was based on a current method being used for extracting dioxins with the Thermo Scientific™ Dionex™ ASE 350 Accelerated Solvent Extractor. This transition proved to be very timely and provided more productivity once online.

When compared to using the Thermo Scientific™ Dionex™ ASE 350 Accelerated Solvent Extractor for extracting dioxin samples, three key improvements were introduced to the sample preparation workflow. Extractions performed by the EXTREVA ASE resulted in approximately 50% reduction in extraction times and solvent consumption when compared to the ASE350. Results will show the recoveries, STDEV and RSD of 170mL and 85mL of solvent used for extraction.

Discussion and Conclusion: This accelerated solvent extraction method proved to be a simple to implement and much faster and more productive than the ASE 350 workflow. Great dioxin recoveries, reproducibility and sample throughput were observed at both solvent extraction volumes that were tested. The system was able to minimize solvent consumption using gas assisted accelerated solvent extraction which results in faster concentration times greater sample throughput. The EXTREVA ASE washing function was able to carry out solvent washing in between samples throughout the sample extract flow paths. This is a critical step when working with Dioxins and proved effective to not have any reportable carryover in between samples.

When factoring in evaporation, the EXTREVA ASE provided a hands-off workflow of extraction and evaporation of samples, removing the need for manual quantitative sample transfer. This manual transfer step is a critical step and can result in a reduction of recovery or even sample loss when using other extraction methods that require an extract transfer step. By combining two sample preparation instruments into one, the EXTREVA system performs both extraction and evaporation for dioxins in one seamless operation. Offering the full benefits of automation and an easy “load-and-go” start process, EXTREVA saves time, reduces errors and solvent usage, enables unattended operations, and significantly increases analytical productivity.

References:
POSTERS

Progress in Methods for POPs Analysis

P-145  New Reference Materials for Organohalogen Contaminants

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Introduction: Reference materials (RMs) are homogeneous, stable and value-assigned for constituents of interest. RMs, especially matrix based RMs, are key components for developing and validating new analytical methods and for evaluating and harmonizing values from established methods. The National Institute of Standards and Technology (NIST) is the metrology agency of the United States and is responsible for providing tools, such as RMs, to support measurements in many areas including the environment and human health. NIST is continually creating new RMs both in response to evolving needs and to replace RMs that have been exhausted. NIST has several new environmental and food RMs in production or recently released that support the monitoring of per- and polyfluorinated alkyl substances (PFAS) and other organohalogen contaminants.

Materials and Methods: Materials were sourced from a variety of locations depending on the intended use. Contaminated soils (sandy and clay soils) were collected from two locations in the United States to yield soils containing different amounts and assemblages of per- and polyfluorinated alkyl substances (PFAS). A fish material consisting of adult lake trout was collected from Lake Ontario (Great Lakes fish). Food and animals feed materials (spinach, pork, beef, corn silage, cow milk, and chicken eggs) were collected from PFAS-contaminated farms. Aqueous film forming foams (AFFF; four total) were dilutions of off-the-shelf materials that were not extracted prior to value assignment. Soils were air-dried, jet milled and irradiated prior to bottling. Biological materials were either cryohomogenized or freeze dried, depending on the material, prior to bottling. A variety of extraction and cleanup methods were used depending on the matrix. For example, the Great Lakes fish material was extracted by pressurized fluid extraction and cleaned up using a combination of size exclusion chromatography and solid phase extraction. All materials were value-assigned for individual PFAS and the Great Lakes fish material will also be value assigned for a variety of other organohalogen contaminants such as PCBs, flame retardants and organochlorine pesticides. Measurements were done using gas and/or liquid chromatography mass spectrometry.

Results: The four AFFF materials are now available for purchase from NIST and have assigned values for up to 17 individual PFAS. Highest concentrations for individual PFAS ranged from 255 mg/g ± 37 mg/g for 6:2 fluorotelomer sulfonamide betaine in reference material (RM) 8693 Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous Film-Forming Foams (AFFF) Formulation IV to 0.056 mg/g ± 0.0019 mg/g for 6:2 fluorotelomer sulfonic acid in RM 8691 Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous Film-Forming Foams (AFFF) Formulation II (Reiner et al. 2023). All other materials have been homogenized and are in different stages in the value assignment process. Of the biological matrix materials, the fish material is furthest along having been value assigned for PFAS and is in the process of being value assigned for other organic contaminants.

Discussion and Conclusion: The reference materials with values for organohalogen contaminants in production or recently produced add to the list of other environmental matrix reference materials already available from NIST. In addition, NIST will soon be producing a candidate reference material for PFAS in drinking water and has plans to upgrade existing materials with SI-traceable concentration values.

Acknowledgments: The authors wish to thank the United States Geological Survey and for fish collection and the United States Department of Defense for providing AFFF materials, soils and financial support.

References:
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**Introduction**: Analysis of polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and Organochlorine Pesticides (OCPs) in sediment requires both extraction and elaborate cleanup of samples. Traditional Soxhlet extraction can take up to 36 hours and manual cleanup of legacy POPs such as PCBs and PBDEs can take several days. Automation of both extraction and cleanup can go a long way towards drastically increasing turnover of these environmental samples.

Existing automated Pressurized Liquid Extraction (PLE) and recently developed low-cost automated cleanup equipment (Auto-EZPrep™) were used to analyze several sediment samples for PCBs, PBDEs, and OCPs. Results are shown below.

**Material and Methods**: 1-2 g of sediment was mixed with Hydromatrix®, spiked with (13C) surrogates and placed in a stainless-steel extraction cell capped with Teflon end caps. The extraction cells were loaded onto the PLE system and extracted with a mixture of 50% dichloromethane and 50% hexane. The cells were pressurized to 1500 psi, heated at 120 °C for 20 min, cooled to ambient temperature, flushed with extraction solvent, and purged with nitrogen gas. The samples collected in glass tubes were then reduced in volume under a nitrogen flow and exchanged to hexane.

Cleanup was carried out with either acidic silica and alumina columns for PCBs/PBDEs or with Florisil for OCPs. The automated system uses a 6-channel multi-pump and a sample processing module with valving. The multi-pump can support up to 6 solvents but for the purpose of this work was run with hexane and 10% dichloromethane in hexane (PCBs/PBDEs) or hexane and 25% DCM in hexane (OCPs). The multi-pump can be set for flows between 1.0-15.0 mL/min and has electronic read-outs for back pressure of the various channels. Each channel can be enabled or turned off for the various cleanup runs.

An important feature is the use of certified columns packed in a clean room, greatly reducing the risk of cross-contamination from the laboratory background. The system itself is closed, which also reduces this risk.

After cleanup the samples were reduced in volume to either 20 uL (PCB/PBDE) or 1 mL (OCPs) and analyzed on an Agilent 7010B GC/MS Triple Quad system.

**Results**: 1-2 g sediment extraction and cleanup. 13C recoveries (PCB/PBDE) and native recoveries (OCPs) in percent.

<table>
<thead>
<tr>
<th>BDE</th>
<th>PCB Number</th>
<th>%</th>
<th>Alpha-BHC</th>
<th>%</th>
<th>Beta-BHC</th>
<th>%</th>
<th>Gamma-BHC</th>
<th>%</th>
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<tbody>
<tr>
<td>BDE-28</td>
<td>PCB-81</td>
<td>96</td>
<td>Alpha-BHC</td>
<td>82</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>BDE-47</td>
<td>PCB-77</td>
<td>92</td>
<td>Beta-BHC</td>
<td>93</td>
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<td></td>
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<tr>
<td>BDE-99</td>
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<td>115</td>
<td>Gamma-BHC</td>
<td>84</td>
<td></td>
<td></td>
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<tr>
<td>BDE-100</td>
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<td>87</td>
<td>Deka-BHC</td>
<td>101</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>BDE-153</td>
<td>PCB-114</td>
<td>107</td>
<td>Heptachlor</td>
<td>80</td>
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<td></td>
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<tr>
<td>BDE-154</td>
<td>PCB-105</td>
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<td>Aldrin</td>
<td>81</td>
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<tr>
<td>BDE-183</td>
<td>PCB-126</td>
<td>102</td>
<td>Heptachlor Epoxy</td>
<td>90</td>
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<tr>
<td>BDE-209</td>
<td>PCB-167</td>
<td>86</td>
<td>Endosulfan I</td>
<td>87</td>
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<tr>
<td>PCB-156</td>
<td>81</td>
<td>4, 4-DDE</td>
<td>85</td>
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<td>PCB-157</td>
<td>87</td>
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<td>PCB-169</td>
<td>105</td>
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<td>74</td>
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</table>

**Conclusion**: Automation of extraction and cleanup greatly speeds up sample processing for both PCBs/PBDEs and OCPs. Both steps combined give very good recoveries for both categories. The automated cleanup system is less expensive than other comparable systems. Extraction can be done in 1 hour, followed by extract concentration, and automated cleanup (maximum 1 hour). Further volume reduction and analysis can all be done on the same day.
Progress in Methods for POPs Analysis

P-147 Development of a Method for Determining Column Robustness

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Introduction: A method was developed to determine column lifetime using a National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 1975 diesel particulate extract which contains polycyclic aromatic hydrocarbons (PAHs), nitro-substituted PAHs as well as a variety of other hydrocarbons and non-volatile residues. The goal was to measure column lifetime which can be applied to estimated lifetime of high throughput environmental laboratory columns using the same column lots.

Materials and Methods: Silarylene “5-type” columns were evaluated by high throughput laboratories while columns of the same lot were analyzed by GC-MS using NIST SRM1975. The instrument was calibrated with 94 semi-volatile compounds which included internal standards and surrogates. Following calibration 10 samples of NIST SRM 1975 diluted 1:1 with methylene chloride were injected. In-between injections a tuning mixture was used to measure system performance. This procedure was repeated until maintenance was required which included replacing the inlet liner, septum, gold seal and removing 50 cm of tubing from the head of the columns. The procedure was repeated until system performance could not be maintained or 300 injections were completed.

Results: The testing indicated a column could be manufactured where performance is restored by trimming following repeated exposure to a highly complex sample.

Discussion and Conclusion: Prototype columns exhibited poor calibration stability where the acid response was priming. This condition would require customers to re-calibrate frequently. Pentachlorophenol is a good indicator of column activity and a measure of robustness and could degrade on a typical column with just 90 injections of the sample where failure is measured as a 20% drop in response.
Progress in Methods for POPs Analysis

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Introduction: Per- and polyfluoralkyl substances or PFAS or synthetic organofluorine chemicals. PFAS are widely used as they have unique desirable properties. Scientists and governments around the world have recognized the harmful effects of PFAS on human health and the environment. The chemical family contains thousands of different PFAS. Methods of analysis, especially those used for enforcement and control, are preferably SI-traceable which means specific target analytes are measured and reported. These target-methods usually contain between 40 to 60 different PFAS compounds, which is far below the actual number of PFAS that is expected to be circulating. A number of studies has demonstrated that for some environmental compartments, only a small number and amount of PFAS is measured by target analysis. This may result in a significant underestimation of the actual PFAS-content. So-called sum-parameter methods are gaining interest from governments and regulators for their comprehensive nature.

Materials and Methods:
To close the gap between the limited number of PFAS measured with the target analysis and the vast number of remaining "unknown" PFAS a so-called Total Oxidizable Precursor Assay (TOPA) can be used. In brief, this method degrades and converts all PFAS oxidatively into a limited number of measurable perfluorinated alkyl acids (PFAAs) of which reference compounds are available and which can be quantified. The complex PFAS compound cocktail is reduced to a relatively easy to measure PFAS mixture. This method was already described previously (Houtz and Sedlak, 2012) and should in theory be a fit-for-purpose solution to on the one hand side vast number of different potential analytes and on the other hand the lack of authentic reference compounds to be used for calibration. Available literature has not yet reported a fully validated TOPA method that yields robust data. Nevertheless, TOPA was put forward as an easy solution for a complex challenge. In this study we have implemented and challenged the previously published protocols. It is important to better understand the capacity, capability, precision, and accuracy of the technique. PFAS quantification was done following the official Flemish PFAS method (WAC/IV/A/025).

Results: The different TOPA method parameters were challenged and further optimized. A positive oxidation control (PFOSA) was used to monitor the conversion efficiency of the TOP assay. It seems that there were high variations between days and that PFOSA was not always completely transformed. In some cases, there was a rest fraction left, ranging from 0 to 30%. Despite all the efforts that were taken, we were not able to transform PFOSA completely or in a reproducible manner. The robustness of the method was lacking to complete a full validation. The only way to move forward is to work with a limit or to set a boundary. If the difference in mass balance (PFCAs) is higher than 20% (before and after TOPA) it only can confirm the presence of unknown precursors.

Discussion and Conclusion: Despite the limits of the TOP assay with respect to conversion efficiency and matrix effect, it has proven a helpful tool to get a helicopter view of complex samples that might be loaded with out-of-the-ordinary-PFAS. The sometimes-low conversion efficiency to measurable and quantifiable analytes leads to an underestimation of the total PFAS content. Nevertheless TOPA can be a useful complementary analysis to any other target method data set, as the outcome can verify whether the target scope gives a sufficiently wide coverage of PFAS measured. Large discrepancies between target data sets and TOPA-data set indicate insufficient compound coverage of the target method. We believe that TOPA can have an added value in the analytical PFAS field, but its applicability is restricted by clear borders and limitation. Expectations should be well managed before application.

Acknowledgments: This study was partially commissioned by the Flemish Government, Department Environment (DO; Department Omgeving). The authors acknowledge Griet Schockaert the interest in the topic and providing funding.

References:
Progress in Methods for POPs Analysis

P-149 Evaluation of triple quadrupole GC-MS/MS on the measurement of polybrominated diphenyl ethers (PBDEs), organochlorine pesticides (OCPs), and polychlorinated biphenyls (PCBs)

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Introduction: Many persistent organic pollutants (POPs) have been routinely measured on gas chromatograph (GC) magnetic sector high-resolution mass spectrometers (HRMS); however, the size, cost, and upkeep of GC-HRMS instruments can make POP analysis a daunting prospect. Recently, new methods for triple-quadrupole GC-mass spectrometers (GC-MS/MS) have been published showing the viability of the technique for regulatory analysis. The lower cost and operation of GC-MS/MS instruments makes them an attractive option for routine analysis, and they are already used in other fields for such purposes. In this study, the viability of using a GC-MS/MS with a short collision cell for analysis of polybrominated diphenyl ethers (PBDEs), organochlorine pesticides (OCPs), and polychlorinated biphenyls (PCBs) was investigated. While other studies have already investigated OCP and PCB analysis using a short collision cell, we sought to expand on these methods to show what is possible using GC-MS/MS.

Materials and Methods: Both native and 13C-labelled standard solutions of PCBs, OCPs, and PBDEs were purchased commercially. Concentrations of 10, 5, 2, 1, 0.5, 0.2, 0.1, and 0.05 ppb were prepared in hexane for each chemical class to test calibration. Measurements were done on a triple quadrupole MS equipped with a GC and a short collision cell. Injections of 2 µL of sample were done using pulsed-splitless injection. Five replicates of each concentration were measured for statistics.

Results: Detector saturation was observed for almost all components at 10 ppb, so this concentration was excluded from calibrations curves. An RFF curve was used to evaluate response. For OCP and PCB compounds, most RFF CV values were less than 10%. Mirex, oxychlorodane, trans-chlordane, and PCB-8 and PCB-81 responded better to linear curves ($R^2 < 0.99$). Internal standards for PCBs and OCPs had RSDs < 15% and < 9%, respectively, except PCB-209 (RSD 15%). For PBDEs, linear response ($R^2 < 0.99$) was generally better than RFF. Relative standard deviation was less than 12% except for PBDE-209 (RSD 16.5%). Qualifying ion ratios were calculated using five replicates at 1 ppb, with RSD values of < 6% for all PCBs and OCPs except oxychlorodane (11%). For PBDEs, RSD values were generally less than 10%, but the higher-substituted compounds saw RSD values as high as 38%. Retention time values were all within 0.05 min of the expected time.

Discussion and Conclusion: Performance of most compounds was excellent using GC-MS/MS. Some compounds that did not perform as well may benefit from further transition optimization studies. In some cases, using the most intense transitions may not translate to the best data based on matrix interferences or interferences from other compounds. All PCB and OCP compounds were observed at their lowest concentration level (0.05 ppb); however, some peaks may be pushing the limits of the instrument. Most PBDE compounds were observed at 0.05 ppb, however, PBDEs 206, 207, and 209 were not observed until 0.2 ppb, highlighting the difficulty of the higher-substituted congeners. While there may be some further improvement requires for some compounds, data supports that the GC-MS/MS is a viable solution for measuring PCBs, OCPs, and PBDEs.

References:
Progress in Methods for POPs Analysis

P-150  Rapid Extraction and Analysis of Drugs in Biological Matrices by Coated Blade Spray-Mass Spectrometry

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Introduction: Coated blade spray (CBS) is a technology that couples ambient ionization-MS with sample preparation by solid phase microextraction (SPME) using a single device. The blade is a thin, flat, conductive substrate with sorbent coating on one end, terminating at a point. Extraction is performed by immersing the sorbent in the sample. Analysis by MS is achieved by adding solvent to the sorbent together with a DC potential, resulting in electrospray ionization. In the present work, the quantitative performance of CBS-MS is investigated and compared to LC-MS for drugs in biological matrices.

Materials and Methods: Coated blades with HLB sorbent and Raptor Biphenyl LC column (1.8 µm, 30 x 2.1 mm) were provided by Restek. Human urine and human plasma were provided by BioIVT. Standards were provided by Cerilliant. All solvents were LC-MS grade. A modified pipetting robot combined with an in-house MS interface was used for extraction and analysis by Thermo TSQ Altis triple quadrupole-MS. LC-MS analysis was performed on a Thermo Vanquish coupled to TSQ Altis.

Urine was spiked with risperidone, ketamine, EDDP, citalopram, LSD, and fentanyl with calibrators at 1, 2.5, 5, 10, 25, 100, 200 ng/mL and QC samples at 15, 75, and 150 ng/mL. Cocaine-d3 and fentanyl-d5 were used as internal standards at 25 ng/mL. Samples were buffered by combining 200 µL of spiked matrix with 100 µL of 5:95 acetonitrile: water, 10 mM K₂CO₃ pH 10.

Plasma was spiked with methotrexate (MTX), 7-hydroxymethotrexate (7-OH MTX), and 2, 4-diamino-N(10)-methylpteroic acid (DAMPA) at 10, 25, 50, 100, 250, 500, 1000, 2500, and 4800 ng/mL. Methotrexate-d3 was used as an internal standard at 100 ng/mL. Samples were buffered by combining 200 µL of spiked plasma with 100 µL of acetonitrile: water, 1.4% formic acid.

Results: Analysis of drugs spiked in urine by CBS-MS provided excellent linearity and accuracy. The following coefficients of determination (R²) obtained: risperidone, 0.997; ketamine, 0.998; EDDP, 0.999; citalopram, 0.998; LSD, 0.999; fentanyl, 0.999.

Analysis of methotrexate and metabolites in plasma by CBS-MS and LC-MS both provided excellent quantitative results. The R² value for MTX by CBS-MS was 0.9977, and LC-MS was 0.9975. Additionally, Passing-Bablok regression between the two methods resulted in an equation of y = 1.019 * x + 3.950. A Bland-Altman plot did not identify any systematic bias between methods across the quantitation range.

Discussion and Conclusion: Analysis of drugs in urine and MTX in plasma by CBS-MS resulted in quantitation performance on-par with LC-MS. These results were achieved with only a minimum of sample preparation, eliminating additional labware waste (SPE plates, centrifuge tubes), laborious and time-consuming steps (centrifugation, filtration, solvent exchange), and solvent usage. Similarly, ambient ionization-MS eliminates mobile phase waste and the time required for LC.

CBS has demonstrated capability of analyzing a wide range of compounds and delivering quantitative performance on par with LC-MS. The workflows involved are greatly simplified in comparison to other SPE techniques and minimize waste. Much of the work to date with CBS has focused of screening/quantitation of small molecules in biological matrices. Future work for the technology includes development of additional sorbent chemistries (ion exchange, etc.) and a broader range of applications (pesticides, PFAS, herbicides, mycotoxins, persistent organic pollutants).

References:
Progress in Methods for POPs Analysis

P-151 Opportunities offered by ion mobility for the characterisation of complex mixtures of PFAS

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Introduction: As the number of perfluorinated compounds described as present in our environment continues to grow, it is now widely accepted that their determination by targeted methods is an impossible task. The development of comprehensive analytical methods capable of detecting and differentiating a large number of PFAS derivatives is therefore crucial to understanding the extent of their presence in ecosystems and its implications. While the use of non-targeted HRMS-based methods allows for the detection of a larger number of PFAS, the retention times and exact masses thus obtained do not in all cases provide sufficient information for the identification of these compounds in the absence of a reference standard. In this sense, ion mobility mass spectrometry (IM-MS) provides an additional descriptor, the collision cross section (CCS), which is related to the size and shape of the molecules. This work describes the development of a TWIMS-ToF-MS method for the analysis of PFAS in a range of biological matrices.

Materials and Methods: Shellfish and fish samples were extracted with a 0.01 M KOH solution in MeOH and purified using two subsequent SPE steps (CHROMABOND® PFAS and ENVI-CARB™ SPE cartridges). IM-MS analyses were performed on a hybrid quadrupole-TWIMS-orthogonal acceleration time-of-flight mass spectrometer (SYNAPT™ G2-Si, Waters™) using an electrospray ionisation source (ESI) in the negative mode, full scan in the [140–800] m/z range, applying the sensitivity mode (25,000 FWHM) for sample analysis. TWIMS CCS calibration was performed using the Waters™ Major Mix IMS/ToF calibration Kit. Data were analysed using DriftScope and Skyline.

Results: The TW CCS values for 24 priority PFAS were obtained experimentally with values between 108.20–214.9 Å². The reproducibility of these measurements was successfully evaluated over seven weeks and in different matrices, allowing the CCS to be used for PFAS characterisation regardless of the type of sample. The application of the ion mobility spectrum filtering in the analysis of biological matrices resulted in significantly more PFAS being detected (+15%) due to a reduction in background noise in 53% of the cases, elimination of co-eluting interferences in 6% of detections, and false negative (14% of the cases) (Figure 1).

Discussion and Conclusion: For PFAS belonging to the same chemical family –e.g., linear sulphonic acid PFAS–, the CCS shows a strong linear tendency with the perfluorinated chain length, allowing predicting the CCS of a compound given the known CCS of structures belonging to the same chemical family. Therefore, the use of IM-MS to study unknown substances in a non-targeted screening to classify them into a given chemical family is considered a promising application. In addition, the use of ion mobility allows the mobility spectrum to be filtered by extracting only those ions within a certain m/z and drift time range, which may result in cleaner chromatograms that improve analyte identification in complex biological matrices. Thus, IM-MS has the potential to (i) increase the signal-to-noise (S/N) ratio by decreasing the background noise, (ii) remove co-eluting peak interferences and/or signals close to an analyte’s retention time that can affect quantitation and, therefore, (iii) prevent false negatives at lower analyte abundances by reducing the background noise and/or removing co-eluting interferences.

Acknowledgments: Grant FPU18/05113 awarded by the Spanish Ministry of Science, Innovation and Universities

Progress in Methods for POPs Analysis

P-152 Automated Sample Preparation and Measurement for Dioxin Analysis

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Introduction: Dioxins are a class of very toxic compounds found throughout the world in the environment. Equipment sensitivity is of great importance for the analysis of low concentrations of these highly-toxic compounds. Historically, analysis and detection of dioxins was done with magnetic sector-type high-resolution mass spectrometers (HRMS). However, in recent years, the performance of triple quadrupole mass spectrometers (MS/MS) has improved significantly. In addition, the development of the Boosted Efficiency Ion Source (BEIS) offers compound-specific sensitivity up to 4 times greater than previous ion sources and provides accurate quantitation of dioxins at levels comparable to HRMS. Detection limits as low as 20 fg for Tetrachlorodibenzo-p-dioxin (TCDD) were achieved. In this study, we analyzed dioxins in about 250 samples of approximately 40 types of food and animal feed products using a GC-MS/MS with BEIS. We also evaluated the number of analyses possible while maintaining sensitivity at low concentrations in order to verify the durability of the GC-MS/MS instrument.

Materials and Methods:

SpeedExtractor: The SpeedExtractor is an automated instrument used for the parallel extraction of primarily organic compounds from a variety of solid samples. In order to maintain the solvent in a liquid state during the extraction process, the solvent inside the extraction cell is put under pressure. To achieve high recoveries, multiple extraction cycles are usually applied. Once the extraction step is finished, the extracts are cooled down in a cooling unit and flushed into collection vials, which can then be easily evaporated.

GO-EHT is an easy-to-operate automated system offering high throughput as well as the additional advantage of using less solvents and consumables. It provides labs with high-quality extraction as well as high return on investment thanks to an innovative flow path system. Total solvent use is only 110 mL.

GCMS TQ8050 NX: The Shimadzu GCMS-TQ8050 and the MRM method are capable of detection and quantitation of seventeen regulated dioxins with high sensitivity and selectivity.

Discussion and Results: The GC-MS/MS system facilitates the screening and quantitation of low concentration PCDD/Fs in different foodstuffs and animal feed samples. The method showed good linearity, sensitivity, and repeatability. This suggests that the GC-MS/MS system provides a substitute solution for routine screening and quantitation of PCDD/Fs in food and feed, as required by European Union legislation.
Screening and Identification of Novel Contaminants

P-153 Analysis and toxicity of “halomix PAHs” using GC-Orbitrap MS

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Introduction: Polycyclic aromatic hydrocarbons (PAHs) are carcinogenic and mutagenic compounds produced by incomplete combustion of organic materials. Halogenated polycyclic aromatic hydrocarbons (HPAHs), in which halogen atoms are substituted for PAHs, have also been reported to be carcinogenic and are considered emerging persistent organic pollutants. In the current field of environmental analysis, HPAHs have only been analyzed for PAHs halogenated with a single halogen atom, either chlorinated or brominated. In fact, it has been suggested that there are HPAHs (halomix PAHs) in which both chlorine and bromine have been substituted in the maternal skeleton of PAHs. However, quantitative analysis and toxicity assessment of halomix PAHs have not yet been conducted, and they are an unknown risk factor. Therefore, in this study, we synthesized halomix pyrene with Pyrene as the nucleus to prepare a standard substance for quantitative analysis of halomix PAHs. We also established a highly sensitive analytical method using GC-Orbitrap MS and attempted to adapt it to environmental analysis. In addition, we evaluated the toxicity of halomix pyrene based on AhR activity using recombinant yeast.

Materials and Method: 1. Synthesis of halomix (Cl/Br) pyrene

Cl/Br-pyrene was synthesized by substituting chlorine and bromine atoms with Pyrene as the core PAH. Propylene carbonate was used as the reaction solvent, N-chlorosuccinimide (NCS) was added in a prescribed amount, chlorination was carried out at 100 degrees Celsius for 30 minutes, and then N-bromosuccinimide (NBS) was added to the reaction mixture in a prescribed amount under the same conditions. The reaction product was recrystallized with methanol/H₂O, and the precipitate was separated and purified by HPLC.

2. Environmental analysis

As the environmental samples, surface soils, ambient particles, lake water, and meat (beef thigh) were collected in Japan. All samples were extracted and cleaned up by each established method and then analyzed using GC-Orbitrap MS.

3. Toxicity evaluation

AhR activity was examined using the recombinant yeast YCM3 strain; toxicity was evaluated by calculating Relative Potency (REP) with respect to BaP. Cl/Br-pyrene, 1-chloropyrene (ClPy), 1-bromopyrene (BrPy), Dichloropyrene (Cl2Py), and Dibromopyrene (Br2Py) were used as test compounds.

Results and Discussion: The synthesis of Cl/Br-pyrene resulted in the formation of several structural isomers. After purification on a C18 column, the peak fractions corresponding to Cl/Br-pyrene were collected. The purified product was used as a standard substance for GC-Orbitrap MS analysis of each environmental samples, which confirmed the presence of Cl/Br-pyrene in the actual environment. Contaminations of Cl/Br-pyrene in the soils and meat samples were observed in 0.01 to 1.27 ng/g and 0.01 to 0.28 ng/g(dw), respectively.

In addition, Cl/Br-pyrene was detected in the ambient particles (n/d~0.02 pg/m³), but not in the lake water samples. These suggest that Cl/Br-pyrene is ubiquitously present in the environment. Of the correlation analysis between the concentrations of Cl/Br-pyrene and pyrene derivatives, significant correlations were observed formation pathway as that of pyrene derivatives.

When Cl/Br-pyrene was applied to YCM3 strain, AhR activity was confirmed (Figure B). From the dose-response curve, the AhR activity of Cl/Br-pyrene was calculated to have a of 0.17. This value is comparable to that of 1-Chloropyrene and Dichloropyrene, suggesting that the biototoxicity of Cl/Br-pyrene is comparable to that of chlorinated PAHs.

Screening and Identification of Novel Contaminants

P-154  Identification of contaminants of emerging concern in tap water from different countries using supramolecular solvent-based microextraction and suspect screening analysis

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Introduction: Continuous technological and industrial growth leads to the overproduction of new chemicals that are released into the environment. Many studies have shown that wastewater effluents from anthropogenic activities contain tens of thousands of chemicals, highlighting the ineffective removal of these compounds in wastewater treatment plants (WWTPs) [1]. These effluents are discharged directly into different water bodies, reaching rivers, lakes, seas and groundwater, which are normally used as a supply source for the production of drinking water. As in the WWTPs, drinking water treatment plants (DWTPs) also have inefficient methods for removing organic pollutants, so chemicals can reach tap water. Chemical and toxicological information on these new compounds is not available or scarce and they are known as contaminants of emerging concern (CECs). While traditional analytical methods allow the detection and determination of known chemical compounds, the use of high-resolution mass spectrometry (HRMS) allows for non-target analysis to detect and identify thousands of chemical products in a single workflow. This paper proposes the use of supramolecular solvents (SUPRAS) as a method for extracting CECs from drinking water samples from 60 cities in different countries of the world, as well as its identification by suspect screening analysis (SSA) HRMS. This new approach can help to understand the role that drinking water plays in human chemical exposure.

Materials and Methods: Extraction of CECs in tap water samples was carried out following a process of SUPRAS-based microextraction. 1-Hexanol was dissolved in Tetrahydrofuran in 2-mL microtubes. Then, the tap water sample was added to promote the coacervation process. The mixture was vortexed at room temperature and centrifuged to accelerate SUPRAS phase separation from the sample. Finally, an aliquot of the SUPRAS extract containing the analytes was directly injected into the LC-QTOF-HRMS system. The SSA workflow comprised different stages: 1) Features filtering by abundance, blanks subtraction, etc.; 2) Grouping and PCA statistical analysis; 3) Feature annotation (suspect list and spectral libraries); 4) Identification.

Results: The developed SSA method was applied to the analysis of 60 drinking water samples collected from tap water in 12 different countries. As an example, two CECs, ethyl 4-dimethylaminobenzoate and nicotine were detected and identified in tap water from nine different cities (Nove Ligure, Kallo, Oporto, Liege, Santiago de Compostela, Verolanuova, Estepona, Pamplona, Gladbeck). The developed workflow was based on the Schymanski-scale to clarify the level of identification reached for each compound. A score greater than 900 points was obtained as a result and both chemicals were identified with a confidence level of 2A.

Discussion and Conclusion: The identified compounds in this study are in agreement with previous results reported for surface water and tap water samples. Thus, nicotine has been found in surface waters of the Ebro River Delta region in Spain [1], demonstrating the ineffective removal of this substance by water purification treatments in drinking water treatment plants. While several personal care products have been found in the drinking water of different homes in North Carolina [2]. The proposed SSA approach has proven to be a powerful tool to elucidate the presence of new chemicals in tap water that might be routinely monitored.

References:
Screening and Identification of Novel Contaminants

P-155 Comprehensive analysis using LC-QTOF-MS in the water environment of Tokyo, Japan

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Introduction: Japan has experienced several cases of chemical spillage into the environment due to accidents and disasters. However, analysis methods that promptly and appropriately address chemical spills have not been sufficiently established. So, we developed comprehensive analytical techniques using high-performance liquid chromatography-quadrupole-time-of-flight mass spectrometry (LC-QTOF-MS) to prepare for emergency responses to chemical leaks and conducted several analyses using these techniques. In this study, we analyzed river water and industrial wastewater in Tokyo to investigate chemical pollution after establishing a database with LC-QTOF-MS data.

Materials and Methods: The LC-QTOF-MS used in this study was a Vion IMS TOF (HPLC part: ACQUITY UPLC H-Class, Waters). The exact mass information of more than 1,000 chemicals, including PRTR Class I designated chemicals, pesticides, pharmaceuticals, and other chemical substances derived from daily life, was registered in the database built into this system. Subsequently, the collision cross section information, which is a unique physical constant of each substance, was registered by analyzing these reference materials. To analyze the water samples, a surrogate mixture was first added to the sample, which was then allowed to flow at a rate of 10 mL/min through a solid-phase cartridge with Oasis-HLB plus 225 mg (Waters) and Sep pak-AC2 plus (Waters) connected in series. After the sample flowed, the solid-phase cartridges were washed with purified water to remove the remaining salts and dried by centrifugation and nitrogen flow. To elute the chemicals adsorbed in the solid-phase cartridge, HLB and AC2 were disconnected. HLB was flushed with 3 mL of methanol, 3 mL of acetone, and 2 mL of dichloromethane, whereas AC2 was flushed with 4 mL of methanol and 3 mL of acetone using the back-flush method.

After concentrating each chemical to approximately 3 mL with a nitrogen purge, the two were mixed, concentrated again to approximately 0.5 mL, fixed at 1 mL with an 80% methanol solution, and analyzed by LC-QTOF-MS.

Results: Table 1 presents the chemicals detected from the Hayase Bridge on the Shingashi River.

Chemicals measured in the positive mode and detected with a count value of 10,000 or higher were extracted. The detected chemicals predominantly originated from daily life, such as pharmaceuticals. To confirm the quantitative accuracy of this analytical method, imidacloprid-d4 and imazalil-d5 were added as surrogates for the pretreatment of hospital wastewater samples, and their approximate concentrations were calculated. The results were further compared with those obtained from LC-MS/MS quantification of the same samples, where the samples were enriched with 13C or deuterium-labeled substances of each chemical as surrogates (Table 2). There was a nearly 10-fold difference between ibesartan concentrations observed at Hospital A. The reasons for this discrepancy are not comprehensively understood. However, it may be necessary to choose more suitable chemicals as surrogates for quantification. Other chemicals exhibited deviations of less than 2-fold, which is not considered a major obstacle in understanding the actual concentrations in the emergency response.

Discussion and Conclusions: We developed a comprehensive analysis method to accurately identify chemicals by measuring river water and matrix-rich wastewater samples under normal conditions. However, as the chemical effluents into the environment during a disaster can differ from typical conditions, it is crucial to identify the chemicals stored in the Tokyo area, register them in a database, and analyze the wastewater to determine the presence of these chemicals. Through these efforts, we aim to further improve our analytical methods and establish a system to facilitate disaster response in cooperation with government agencies.

Acknowledgments: This research was supported by the Environment Research and Technology Development Funds (JPMEEF18S11712) of the Environmental Restoration and Conservation Agency of Japan.

Table 1 Results of screening for chemicals at the Hayase Bridge

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Mass use or origin</th>
<th>Detector count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diclofenac</td>
<td>Antiprostaglandin</td>
<td>118</td>
</tr>
<tr>
<td>Sulfadiazine</td>
<td>Antimicrobial</td>
<td>71,047</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>Antibiotic</td>
<td>51,756</td>
</tr>
<tr>
<td>Lidocaine</td>
<td>Local anesthetic</td>
<td>45,067</td>
</tr>
<tr>
<td>Epinastine</td>
<td>Antihistamine</td>
<td>37,846</td>
</tr>
<tr>
<td>14-Hydroxyclarithromycin</td>
<td>Metabolite of Clarithromycin</td>
<td>42,474</td>
</tr>
<tr>
<td>Caffeine</td>
<td>Central nervous system stimulant</td>
<td>17,377</td>
</tr>
<tr>
<td>Lofactan</td>
<td>Antihypertensive Agent</td>
<td>22,457</td>
</tr>
<tr>
<td>Diclofenamide</td>
<td>Antiarhythmic medication</td>
<td>13,470</td>
</tr>
<tr>
<td>Aloemassol</td>
<td>Antiarhythmic medication</td>
<td>12,380</td>
</tr>
</tbody>
</table>

Table 2 Comparison between the concentrations of chemicals in hospital effluents quantitated with LC-QTOF-MS and LC-MS/MS : ng/L

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Hospital A Concentration with LC-QTOF-MS</th>
<th>Hospital B Concentration with LC-QTOF-MS</th>
<th>Hospital A Concentration with LC-MS/MS</th>
<th>Hospital B Concentration with LC-MS/MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clarithromycin</td>
<td>118</td>
<td>54</td>
<td>75</td>
<td>55</td>
</tr>
<tr>
<td>14-Hydroxyclarithromycin</td>
<td>160</td>
<td>61</td>
<td>77</td>
<td></td>
</tr>
<tr>
<td>Carbenavastate</td>
<td>2,200</td>
<td>700</td>
<td>1,200</td>
<td>1,000</td>
</tr>
<tr>
<td>Sulindic</td>
<td>5,000</td>
<td>700</td>
<td>680</td>
<td>700</td>
</tr>
<tr>
<td>Telmisartan</td>
<td>1,200</td>
<td>30</td>
<td>89</td>
<td>90</td>
</tr>
<tr>
<td>Losartan</td>
<td>1,200</td>
<td>30</td>
<td>89</td>
<td>90</td>
</tr>
<tr>
<td>Candoxatran</td>
<td>470</td>
<td>85</td>
<td>80</td>
<td>70</td>
</tr>
<tr>
<td>Crotamiton</td>
<td>750</td>
<td>54</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Fexofenadine</td>
<td>5,200</td>
<td>1,200</td>
<td>1,800</td>
<td>1,800</td>
</tr>
<tr>
<td>Epinastine</td>
<td>69</td>
<td>82</td>
<td>68</td>
<td>75</td>
</tr>
</tbody>
</table>
Screening and Identification of Novel Contaminants

P-156  Optimized non-target screening workflow for estimating industrial chemicals discharged from marine industrial facilities in Korea

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Introduction: Anthropogenic activities such as urbanization and industrialization augment the concerns about the occurrence of industrial chemicals in the marine environment. Some industrial chemicals have endocrine disruptors, carcinogens, and mutagens to marine organisms and human health; thus, continuous monitoring is required. In general, pollutants in the marine environment have mainly been monitored based on target analysis. However, target analysis is not enough to monitor numerous industrial chemicals in the marine environment due to the limitation of simultaneous chemical analysis volume. Accordingly, it is necessary to apply new scientific methodologies such as non-target analysis techniques, which can simultaneously screen multiple chemicals, but these techniques inevitably entail a data post-processing process for massive chemical information. The post-processing of non-targeted screening data requires significant labor and time, necessitating the development of novel data processing techniques to overcome these challenges. This study aims to develop an optimized non-target screening workflow for estimating industrial chemicals discharged from marine industrial facilities in Korea.

Materials and Methods: 23 wastewater samples were collected from seven industrial facilities adjacent to the Korean coast. Industrial chemicals in the wastewater were analyzed using gas chromatography coupled with a high-resolution time-of-flight mass spectrometer (GC-TOF-MS). The data post-processing technique to estimate industrial chemicals from non-target screening data was established in the open-source R package.

Results: On average, up to 5,600 features were detected in the wastewater samples from non-target screening. After manual data post-processing, an average of 100 industrial chemicals were identified. On the other hand, the automatic data post-processing identified an average of 80 industrial chemicals. Comparing these two processes, emerging contaminants such as organophosphorus flame retardants (OPFRs), siloxanes, phthalates, and alternative plasticizers (APs) were commonly detected, and the matching rate of the detected substances was more than 75%. More than 200 industrial chemicals were detected from 8 marine industrial facilities, and these chemicals are composed of hazardous and noxious substances (HNS), emerging contaminants, industrial solvents, polycyclic aromatic hydrocarbons (PAHs), etc. By type of marine industrial facilities, an average of 60 to 110 industrial chemicals were detected, and the number of detected substances was highest in the automobile manufacturing and petrochemical industries. Detected industrial chemicals showed a detection frequency ranging from 4% to 93%, and phthalates and siloxanes had a higher detection rate than other chemicals.

Discussion and Conclusion: The non-target screening process was optimized and applied to wastewater from marine industrial facilities. A good agreement with manual non-target data post-processing suggests that the automatic data post-processing established in this study is working well in real samples. The non-targeted screening confirmed the discharge of various industrial chemicals, including HNS, known to cause potential acute toxicity, from marine industrial facilities, highlighting the importance of multi-component simultaneous analysis-based monitoring. The high detection frequency of phthalates and siloxanes in wastewater may be related to their wide use in industrial activities, implying the need for comprehensive investigations of their potential risk to the marine environment.

Acknowledgments: This research was supported by Korea Institute of Marine Science & Technology Promotion(KIMST) funded by the Ministry of Oceans and Fisheries, Korea (20210660, 'Development of Technology for Impact Assessment and Management of HNS discharged from Marine Industrial Facilities').
Posters

Screening and Identification of Novel Contaminants

P-157 Identification and characterisation of quaternary ammonium compounds in Flemish indoor dust by ion-mobility high-resolution mass spectrometry.

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Introduction: Quaternary ammonium compounds (QACs) are a class of surface-active substances which are commonly used in disinfecting and cleaning products. Thereby, alkyl trimethylammonium compounds (ATMACs), benzyl alkyl dimethylammonium compounds (BACs) and dialkyl dimethylammonium compounds (DDACs) are the most frequently used QAC classes. The COVID-19 pandemic has led to a substantial increase in their use, resulting in a risk of human exposure to these compounds. A recent study quantified 19 QACs in indoor dust samples collected in the United States observing significant increases in QAC concentrations between samples collected before and during the COVID-19 pandemic and identifying the ingestion and inhalation of dust as an important human exposure route [1]. Studies on the occurrence of QACs in European dust samples are lacking.

The presented study introduces a targeted and suspect screening analysis of indoor dust samples by liquid chromatography-ion-mobility high resolution mass spectrometry (LC-IM-HRMS) aiming at a comprehensive characterization of QACs and an estimation of potential health risks following human exposure to these compounds.

Materials and Methods: Indoor dust samples (n=46), collected at 40 different locations in Flanders, Belgium, were sieved and directly extracted twice with MeOH. Extracts were analyzed by liquid chromatography coupled to LC-IM-HRMS. Calibration curves of 21 QACs were used for confirmation and semi-quantification of targeted QACs. Suspect screening analysis included matching the obtained data with a suspect list containing > 350 entities including QACs from the three investigated classes and other quaternary ammonium surfactants. Each identified suspect was semi-quantified using a calibration curve of a targeted QAC which showed the highest possible structural similarity. (Semi-)quantified concentrations allowed the calculations of estimated daily intakes (EDI) and hazard quotients (HQ) based on different exposure models. IM derived collision cross section (CCS) values obtained for suspects were compared with CCS-m/z trendlines of the targeted QACs whereby an alignment between the suspects’ CCS value and the targets’ trendline was considered as an additional identification parameter adding confidence to compound annotation.

Results: All targeted QACs (n = 21) were detected in indoor dust samples with detection frequencies (DFs) ranging between 4.2 and 100%, while 15 QACs showed detection frequencies > 90%. Semi-quantified concentrations of individual QACs showed a maximum of 32 µg/g with a median ∼QAC concentration of 13 µg/g. As the applied quantification method uses HRMS derived raw data and was not fully validated, the presented concentrations have to be interpreted with care. Nevertheless, most abundant QACs matched the patterns reported in dust collected in the United States. HQs obtained from semi-quantified concentrations of targeted compounds were all < 1 not indicating potential health risks. However, this approach does not take other exposure routes and sources into consideration. Suspect screening allowed the identification of 17 additional QACs. A dialkyl dimethyl ammonium compound with mixed chain lengths (C16:C18) was characterized as a major QAC homologue with a maximum semi-quantified concentration of 25 µg/g. Additionally, dimethyl ethyl alkyl ammonium compounds (DEACs), including side chains of 12 to 18 carbon atoms were identified as a novel homologue series.

CCS-m/z trendlines were established from the CCS values obtained for the targeted QACs whereby a clear distinction between the three QAC classes was possible. CCS values obtained for suspect QACs which were structurally similar to targeted QACs aligned with the established trendlines thereby confirming compound identification.

Discussion and Conclusion: The obtained targeted results unequivocally confirm the ubiquitous presence of known QAC classes in the European indoor environment at µg/g concentration levels. The high number of identified suspects indicates a high structural variability of these compounds and points out the added value of the applied suspect screening approach. Both results call for more European studies on potential human exposure to these compounds.

The established reference CCS values and CCS-m/z trendlines served as an additional identification parameter and presented an approach which allows the use of IM-HRMS derived data to increase the confidence in the assignment of suspect compounds.

Screening and Identification of Novel Contaminants

P-158 Sunscreen Agents and Ultra-Violet Filters in Freshwater Sediments: TERRAChem method development and validation

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Introduction: Chemical sunscreen agents (SSAs) and ultra-violet filters (UVFs) are extremely common and extensively used in the form of personal care products (hair sprays, foundations, lip balms, nail polishes, shampoos, sunscreen lotions, etc.) to absorb UV and visible sun rays and to prevent sunburn, but also as UV light absorbers and stabilisers in plastics, coatings, and adhesives. These chemicals are continuously released in effluent from wastewater treatment plants and as such have been termed pseudo-persistent despite their relatively short environmental half-lives. Consequently, they have been reported to accumulate in freshwater and marine sediments, and aquatic biota1,2. SSAs/UVFs are known or suspected endocrine disruptors and can be toxic to aquatic organisms. In Ireland, there is a dearth of knowledge regarding the presence and prevalence of these emerging contaminants in the environment despite their extensive use. It is therefore unclear how pervasive these chemicals are in the Irish environment and whether they are present at levels which may pose a risk to the environment or living organisms. The TERRAChem project aims to fill this knowledge-gap by implementing a nationwide assessment in Ireland of a wide range of emerging contaminants (including SSAs/UVFs) in sediments and sewage sludge and evaluate the risks associated with their presence. Here, we quantitatively determine five SSAs/UVFs (enzacamene, oxybenzone, octocrylene, octyl 4-methoxycinnamate, and homosalate) in archived riverine sediments from the UK, as well as in a certified reference material (ERM-CC537a) for freshwater sediment. The results presented represent the initial findings of the method development and validation for the TERRAChem project for SSAs/UVFs in sediments.

Methods and Materials: Analyte concentrations and absolute recoveries of SSAs/UVFs were determined using standard additions analysis. Six 500 mg subsamples of each lyophilised sediment sample were taken for analysis, including three sample replicates and three sample replicates spiked with 50 ng of native analytes (100 ng/g). The analytes were extracted from the sediment using 10 mL acetonitrile and H2O (1:1) and cleaned using QuEChERS salts (4g MgSO4 and 1g NaCl), followed by solid phase extraction (PRiME HLB, 60 mg) eluted by acetonitrile. Cleaned extracts were analysed using a UHPLC-TOF-MS/MS (Sciex 5600+).

Results: Mean concentrations of octocrylene in Severn river sediment were 2.8±0.96 ng/g, while in ERM-CC537a, mean concentrations of octocrylene were 2.9±0.42 ng/g. Enzacamene, oxybenzone (BP-3), octyl 4-methoxycinnamate and homosalate were not detected in either the UK river or CRM sediments. Absolute recoveries of native SSAs/UVFs in the spiked subsamples from standard additions analysis (n=6) ranged from 53 – 63% for enzacamene, 89 – 126% for oxybenzone, 62 – 95% for octocrylene, 33 – 53% for octyl 4-methoxycinnamate, and 57 – 89% for homosalate. The calculated sample detection limits (SDLs) were 0.37 ng/g, 0.41 ng/g, 0.49 ng/g, 0.010 ng/g, and 0.20 ng/g for enzacamene, oxybenzone, octocrylene, octyl 4-methoxycinnamate, and homosalate, respectively.

Discussion and Conclusion: Concentrations in the Severn river sediment and ERM-CC537a samples are fairly low and are consistent with what has been previously reported for octocrylene in Norwegian and Chesapeake Bay, VA, USA sediments1,2. The absolute recoveries and SDLs derived from both UK river sediments and the ERM-CC537a sediment show that the method is appropriate for use in the TERRAChem project.

Acknowledgements: This project (TERRAChem, reference 2021-HE-1056) is funded under the EPA Research Programme 2021-2030. The EPA Research Programme is a Government of Ireland initiative funded by the Department of Communications, Climate Action, and Environment.

References:
Status and Perspective on Waste Management of POPs

P-159  PCDDs/DFs Emission Characteristics from Intermittently Operated Small-scale Waste Incineration Facilities in Korea

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Introduction:
PCDDs/DFs are generated from incineration processes such as waste incineration facilities, sewage sludge, automobiles, organic chlorine compound production processes, smelting processes, and among them, it is known that they are mainly generated in the incineration process of municipal solid waste. As PCDDs/DFs are already regulated by Persistent Pollutant Control Act in Korea, legal standards are well regulated in large-scale waste incineration facilities. But high concentrations of dioxins are still being emitted from some small intermittently operated waste incineration facilities operating less than 8 hours a day. In this study, we tried to examine the emission behavior of PCDDs/DFs by the waste incineration process in small batch type waste incineration facilities.

Materials and Methods:
In this study, 2 small-scale waste incineration facilities that operated intermittently (less than 8 hours a day) were studied in 4 stages which are divided into heating stage, waste input stage, stabilization stage, extinguishing stage and the concentration of PCDDs/DFs in exhaust gas in each stage were measured. And also, the concentrations of O₂, CO, NOₓ, and SOₓ in the exhaust gas were measured to check the oxygen concentration correction and air pollutant concentration distribution. In addition, the control and emission characteristics of dioxins by the prevention facility were also identified through simultaneous measurement of the front and rear ends of the prevention facility. Meanwhile, dioxin analysis was performed by collecting the residue accumulated at the bottom of the stack.

Results:
PCDDs/DFs emission by stage showed very high concentrations of 0.204~74.753 ng I-TEQ/Sm³ and 21.545~96.143 ng I-TEQ/Sm³ for each facility from the heating stage to extinguishing stage, respectively.

The PCDDs/DFs removal rate in the prevention facility showed an average removal rate of 96.6% in the initial temperature rising stage, but as the incineration process progressed, the removal rate rapidly decreased, and it was investigated that the removal rate dropped sharply to 27.3% in the extinguishing stage. It is known that the input of activated carbon is very important for the removal of dioxin from the exhaust gas, but it was determined that the activated carbon was not continuously input during the incineration process in target facilities, but was only temporarily input at the beginning of operation. On the other hands, the PCDDs/DFs concentrations in the solid residue at the bottom of stack were 26.804 ng I-TEQ/g and 1,012.530 ng-TEQ/g, respectively. Facility with high concentration of PCDDs/DFs in solid residues also showed very high concentration of PCDDs/DFs in the stack exhaust gas, which is considered to be that the concentrated dioxins in the residues could also affect the exhaust gases.

Discussion and Conclusion:
In Korea, the contribution rate of dioxin emissions from small-scale waste incineration facilities is very low, less than 6% of the total. However, the rate of exceeding the emission limit and the emission concentration are very high compared to large facilities, and have been increasing recently.

In this study, the PCDDs/DFs concentration in the exhaust gas from heating stage to extinguishing stage were analyzed, and it was confirmed that high concentrations of PCDDs/DFs could be emitted during the repeated heating and extinguishing process. In order to reduce high-concentration PCDDs/DFs emissions at small-scale waste incineration facilities with relatively poor operating conditions compared to large-scale waste incineration facilities, various policy measures should be introduced. And it is suggested that the large-scale system should be institutionally introduced to enable stable waste incineration through continuous operation.

References:
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1 Introduction

The Stockholm Convention on Persistent Organic Pollutants (POPs) aims to protect human health and the environment from POPs. The POPs Convention (Stockholm Convention on POPs) aims to protect human health and the environment from the production and use of POPs and stipulates the elimination, restriction, reduction of emissions, and disposal of waste containing POPs. Therefore, it is necessary to consider appropriate disposal methods for substances that have been added to the scope of the POPs Convention. Long-chain perfluoro carboxylic acids (PFCAs) have been decided to be considered for addition to the POPs Convention in 2022 (C9-C21) and the REACH regulation in 2021 (C9-C14).

It has been reported that the properties of perfluoro carboxylic acids (PFCAs) change significantly with a slight difference in the number of carbons1, and it is necessary to confirm whether the number of carbons affects the properties of PFCAs in incineration. It is necessary to confirm whether or not there is an effect of carbon number on the properties of PFCAs during incineration.

Although the incineration behavior of per- and polyfluoroalkyl substances (PFAS) pure substances has been reported2, incineration of actual products containing PFAS has not been conducted. It is difficult to guess the destruction behavior at this time by incineration of pure substances only. In the incineration test of foam fire extinguishing agents containing PFAS, it is difficult to perform the desired quantitative evaluation due to the extremely low concentration of PFAS in the product.

The objective of this study was to understand the destruction behavior by conducting incineration tests of fluorinated POPs (including pure long-chain PFCAs and a next-generation POPs) for which there are few research cases, and an actual product by adding PFAS. Incineration of perfluorooctanoic acid (PFOA) pure material was also conducted with baffles installed to investigate ways to further increase the destruction efficiency.

2 Materials and Method

2.1 Sample

Table1: Incinerated sample in long-chain PFCAs.

<table>
<thead>
<tr>
<th>name</th>
<th>abbreviation</th>
<th>carbon number</th>
<th>Purity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perfluorononanoic acid</td>
<td>PFNA</td>
<td>9</td>
<td>98%</td>
</tr>
<tr>
<td>Perfluorodecanoic acid</td>
<td>PFDA</td>
<td>10</td>
<td>100%</td>
</tr>
<tr>
<td>Perfluoroundecanoic acid</td>
<td>PFDnDA</td>
<td>11</td>
<td>95%</td>
</tr>
<tr>
<td>Perfluorododecanoic acid</td>
<td>PFDoDA</td>
<td>12</td>
<td>96%</td>
</tr>
<tr>
<td>Perfluorotetradecanoic acid</td>
<td>PFTeDA</td>
<td>14</td>
<td>100%</td>
</tr>
<tr>
<td>Perfluorooctadecanoic acid</td>
<td>PFODa</td>
<td>18</td>
<td>95%</td>
</tr>
</tbody>
</table>

*Results from instrumental analysis

The substances used as long-chain PFCAs are shown in Table 1. The actual product was the firefighting foam shown in Table 2, to which PFHxS, PFOS, and PFOA were added at about 3% each. The results of instrumental analysis showed that PFHxS, PFOS, and PFOA were 2.835%, 2.234%, and 2.234%, respectively. PFOA was used in the baffle incineration test, and the results of instrumental analysis showed a purity of 100%.

Table2: Composition of Firefighting Foam.

<table>
<thead>
<tr>
<th>inclusion</th>
<th>mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFBS</td>
<td>170</td>
</tr>
<tr>
<td>PFHxS</td>
<td>760</td>
</tr>
<tr>
<td>PFHpS</td>
<td>140</td>
</tr>
<tr>
<td>PFOS</td>
<td>9200</td>
</tr>
<tr>
<td>PFDS</td>
<td>2</td>
</tr>
<tr>
<td>PFBA</td>
<td>28</td>
</tr>
<tr>
<td>PFPeA</td>
<td>27</td>
</tr>
<tr>
<td>PFHxA</td>
<td>100</td>
</tr>
<tr>
<td>PFHpA</td>
<td>31</td>
</tr>
<tr>
<td>PFOA</td>
<td>140</td>
</tr>
<tr>
<td>PFDA</td>
<td>0.058</td>
</tr>
<tr>
<td>PFUnDA</td>
<td>0.025</td>
</tr>
<tr>
<td>PFEESA</td>
<td>0.72</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>inclusion</th>
<th>mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>GenX</td>
<td>0.68</td>
</tr>
<tr>
<td>PFMOPA</td>
<td>0.13</td>
</tr>
<tr>
<td>PFMOBA</td>
<td>0.083</td>
</tr>
<tr>
<td>FBSA</td>
<td>5.5</td>
</tr>
<tr>
<td>FHxSA</td>
<td>21</td>
</tr>
<tr>
<td>FOSA</td>
<td>1.9</td>
</tr>
<tr>
<td>N-MeFBSA</td>
<td>0.32</td>
</tr>
<tr>
<td>N-MeFOSA</td>
<td>0.38</td>
</tr>
<tr>
<td>N-MeFBSE</td>
<td>0.91</td>
</tr>
<tr>
<td>N-MeFSE</td>
<td>3.7</td>
</tr>
<tr>
<td>N-EtFSE</td>
<td>1.5</td>
</tr>
<tr>
<td>N-AP-FHxSA</td>
<td>150</td>
</tr>
<tr>
<td>N-TAmP-FHxSA</td>
<td>260</td>
</tr>
</tbody>
</table>
2.2 Incineration Test

The lab-scale furnace shown in Figure 1 is designed to control heating temperature and gas flow rate. A quartz boat was installed in the core tube upstream of the furnace, and a glass filter (GF), adsorbent (PUF/XAD), empty bottle, two toluene traps, and two NaOH (0.1 M) traps were connected downstream to collect destruction products. Approximately 10 mg of sample was put into each of the 10 circular grooves on the quartz boat, for a total of 100 mg. For the baffled incineration test, two types of baffles were used: half-moon and circular. The half-moon baffle is a semicircle with a radius of 1.05 cm, and the circular baffle is hollow in the middle. This baffle was placed 4 cm downstream from the quartz boat. The incineration test was conducted at 850°C, residence time of 2 seconds, pure air gas, and sample feed rate of 1 cm/30 sec.

2.3 Pretreatment and Instrumental Analysis

After incineration, the samples were collected in nine different media: upstream of the core tube, adhered materials (downstream of the core tube, each media connection, and empty bottle), quartz boat, glass filter (GF), adsorbent (XAD and PUF), toluene first and second stage, NaOH first and second stage. After pretreatment, instrumental analysis was performed using a liquid chromatography-time of flight mass spectrometer (LC-ToF/MS) to quantify this sample and byproducts by media. Eighteen PFAS (PFBS, PFHxS, PFHpS, PFOS, PFOS, PFBA, PFPeA, PFHxS, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTeDA, PFHxDA, and PFOcDA) were included in the determination.

2.4 Destruction Efficiency

After quantification, the destruction efficiency and residual percentage were calculated using the following equation.

\[
\text{Destruction efficiency} \, [\%] = \left( \frac{A_i - A_{s+l+g}}{A_i} \right) \times 100
\]

\[
\text{Residual percentage} \, [\%] = 100 - \text{destruction efficiency}
\]

\(A_i\): initial amount of material (g), \(A_{s+l+g}\): amount of material in solid, liquid and gas phase after incineration (g).

In order to evaluate the destruction behavior after the incinerator, the data upstream of the core tube shall not be included in the calculation of the destruction rate. In the case where a substance could not be quantified in a medium, the lower limit of quantification was used to estimate its approximate amount by calculating the destruction efficiency.

3 Results and Discussion

Figure 2: Residual percentage of long-chain PFCAs after incineration at 850°C.
Status and Perspective on Waste Management of POPs

P-160  Destruction behaviors of fluorinated POPs by incineration: Evaluation of carbon chain length, difference between pure substances and products, and baffle effect

![Figure 3: Distribution behavior of pure substances and PFAS-added firefighting foam products after incineration. *Data of pure substances are referred from our previous study.](image)

![Figure 4: Residual percentage of PFOA with baffle after incineration at 850°C.](image)

### 3.1 Destruction Efficiency

The results of the quantitative analysis of PFCAs pure material by incineration test are shown in Figure 2. The horizontal solid line is drawn based on the destruction rate of 99.999% recommended by the Basel Convention, and a value below this line means that a destruction rate of over 99.999% is achieved. For long-chain PFCAs, the residual percentages were below 0.001% (solid line) under all conditions, indicating that the destruction was sufficiently feasible under the conditions of 850 °C, residence time of 2 s, and pure air gas. Residuals of substances with 10 or more carbons (from PFDA to PF0cDA) were at most about one order of magnitude lower than those of substances with 9 or less carbons, indicating a higher reproducibility of the results. The reason for the lower residuals may be related to the decrease in vapor pressure associated with the increase in the number of carbons; PFOA has a tendency to migrate into exhaust gases. PFCAs with higher carbon numbers have lower vapor pressure, which slows vaporization and prevents migration into exhaust gases, possibly resulting in higher destruction efficiency.

Figure 3 shows the results of the quantitative analysis of PFAS-added firefighting foam by incineration tests, and the residual rates were calculated for each of PFHxS, PFOS, and PFOA contained in the PFAS foam. Here, PFOA in the residue of PFAS-added firefighting foam exceeded 0.001%. This may be due to the fact that high concentrations of PFOA were measured in the instrumental analysis immediately prior to the analysis, and this effect may have been apparent in the results. In this study, this value is treated as a reference value. In addition, both PFHxS and PFOS, which are components of the foam fire extinguishing agent, were below a residual rate of 0.001%. This shows that PFAS-added firefighting foam has almost the same residual rate as each pure substance, and that even the actual product can be destructed by incineration.
The quantitative results of the incineration test of PFOA with baffles are shown in Figure 4. Compared to the results without baffles, the residual percentages of both the half-moon and circular shapes were reduced by approximately one order of magnitude. One possible reason for the decrease in residual percentage is that the installation of baffles caused turbulence in the furnace, which increased the residence time of PFOA in the furnace locally. There was no significant difference in the residual rate between the half-moon type and the round type.

3.2 Distribution Behavior
After incineration of PFAS-added foam extinguishing agent, the percentage of destruction products collected by each medium is shown in the lower part of Figure 3. The blue graph represents the exhaust gas collection part and the black collection part represents the solid residuals; the PFAS foam extinguishing agent and the pure material showed similar distribution behavior with a high percentage of exhaust gas component. It has been reported that PFHxS pure material tends to remain as a solid component. The residual tendency is stronger at higher temperatures (950°C to 1000°C), indicating that the proportion of the exhaust gas component increases in the temperature range of 850°C. The proportion of the exhaust gas component increases at higher temperatures (950°C to 1000°C). The reason for this increase or decrease in the ratio may be that the destruction rate changes with temperature; when PFHxS vaporizes in the furnace, the high temperature of 1000°C may accelerate its destruction, preventing it from migrating to the flue gas collection section. The destruction rate of PFHxS was lower in the case of the foam extinguishing agent with PFAS, which was incinerated at 850°C, resulting in an increase in the percentage of PFHxS in the flue gas collection section.

For the long-chain PFCAs and baffled PFOA, it was not possible to properly evaluate them because the destruction products were below the lower limit of quantification for almost all media.

3.3 By-products
As byproducts of PFTeDA (C14), many substances with similar carbon numbers, such as PFTrDA (C13), PFDoDA (C12), and PFUnDA (C11), were detected upstream of the core tube, and PFTeDA itself was also detected at high values. This is thought to be because PFTeDA vaporized and adhered to the upstream portion of the core tube, and by-products remained undecomposed due to incomplete thermal destruction caused by insufficient temperature. In addition, although not destruction by incineration, photochemical destruction of PFOA suggests carbon chain reduction reactions by decarboxylation, hydroxylation, and hydrolysis. For PFAS-added firefighting foam and baffled PFOA, byproducts (PFHpA, PFBA) due to carbon chain reduction were observed in a small fraction of the samples.

4 Conclusions
This study clarified the destruction behavior of long-chain PFCAs and PFAS-added foam extinguishing agents by conducting incineration tests. The results showed that 99.999% (residual <0.001%) was achieved for each substance under the set conditions, indicating that even the actual product is sufficiently degradable. Among the long-chain PFCAs, substances with higher carbon numbers tended to have lower residual percentages. Baffle incineration tests conducted to examine destruction rate improvement showed that the destruction rate improved by approximately one order of magnitude regardless of the shape of the baffle. This suggests that the longer residence time (in other words, "local retention") due to turbulence in the furnace may have affected the destruction efficiency. In the future, we plan to verify the baffle effect by conducting baffle incineration tests using substances with lower destruction rates than those in past data.

In the distribution behavior of PFAS-added firefighting foam by medium, the ratio in the flue gas collection section tended to be high, showing similar behavior to that of pure substances. Long-chain PFCAs and baffled PFOA could not be properly evaluated because they were below the lower limit of quantification in almost all media. Future studies are needed to clarify the distribution behavior of each medium by intentionally suppressing destruction in a low temperature range (200°C to 700°C).

As a byproduct, a substance with a reduced carbon number of PFCAs was identified, suggesting a destruction pathway of carbon chain reduction by incineration.

Acknowledgments
This work was performed by the Environment Research and Technology Development Fund [JPMEERF20213002, 3-2102(1), (3)] of the Environmental Restoration and Conservation Agency (ERCA) of Japan.

References
Introduction: Di(2-ethylhexyl) phthalate (DEHP) is widely used as a plasticizer in manufacturing plastics. In utero exposure to DEHP may affect the male reproductive system and has been shown to have transgenerational effects. Our previous report has investigated the transgenerational effects of high-dose DEHP exposure on male reproduction (Hsu et al., 2021). However, few studies have reported the transgenerational effects of low-dose DEHP exposure on male reproduction. The present study was to investigate the low-dose DEHP maternal exposure on reproductive functions and epigenetic transgenerational effects in male rat offspring.

Materials and Methods: Pregnant SD rats (F0) were treated through gavage on GD 0 to birth with vehicle control (corn oil), 20 µg/kg/day DEHP, or 200 µg/kg/day DEHP. Only the F0 generation gestating female was exposed directly. Male rats born to rats from the F1 were labeled the F2. Male rats born to rats from the F2 were labeled the F3. The offspring’s body weight, anogenital distance (AGD), anogenital index (AGI), tissues weights, sperm count, motility, morphology, sperm reactive oxygen species (ROS), mitochondrial membrane potential (MMP), DNA fragmentation index (DFI), DNA content analysis of testis cells, serum testosterone and DNA methyltransferases (Dnmts) were measured for all generations. Whole-genome bisulfite sequencing (WGBS) was also examined to analyze sperm DNA methylation status in the F3 generation.

Results: DEHP exposure at 20 µg/kg affected developmental, AGD, AGI, and mean DFI in the F1; sperm morphology in the F2; and ROS, mean DFI, and Dnmt1 in the F3. DEHP exposure at 200 µg/kg affected developmental, body weight, AGI, MMP, and mean DFI in the F1; mean DFI in the F2; and ROS and mean DFI in F3. Compared with the control group, 24 and 27 differentially hypermethylated genes were identified in the groups administered F3 20 µg/kg and 200 µg/kg DEHP, respectively. Moreover, 11 and 18 differentially hypomethylated genes were observed in the groups administered 20 µg/kg and 200 µg/kg DEHP.

Discussion and Conclusion: Several studies have indicated that prenatal exposure to DEHP might induce developmental toxicity, endocrine disruption, and reproductive hazards in a dose-response manner in male offspring (Agarwal et al., 1989; Barakat et al., 2017). The present finding suggests that prenatal low-dose DEHP exposure may epigenetically affect male reproduction through transgenerational effects. In conclusion, pregnant rats exposed to low-dose DEHP might induce reproductive toxicity in male offspring and the transgenerational epigenetic impact in F3.

Acknowledgments: This work was supported by the National Health Research Institutes (NHRI-105A1-PDCO-3316161) and the Ministry of Science and Technology (MOST 109-2221-E-992-043-MY3) of Taiwan.

References:
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1. Introduction:
Pharmaceuticals and/or their metabolites are excreted via urine and feces after administration. Since many compounds cannot be completely eliminated by conventional wastewater treatment plants (WWTPs), they are regularly detected in receiving waters [1,2]. However, to provide a comprehensive picture of the burden of pharmaceuticals on the aquatic environment, special expertise about pharmacokinetics may be necessary to implement reasonable monitoring approaches: Some compounds are extensively metabolized in the human body so that the parent compound is excreted in negligible quantities, while others are even administered as so-called pro-drugs, i.e. molecules with little or no pharmacological activity that must undergo enzymatic and/or chemical transformation in vivo, to release the active compound.

The environmental risk assessment for human medicines in the EU largely ignores metabolism in authorization procedures, following a total residue approach for a conservative exposure prediction in risk characterization [3]. However, metabolites of heavily transformed drugs need to be included in monitoring programs in order to gain profound insights into the respective drug’s burden on the aquatic environment. Information on the identity and extraction fractions of many metabolites is still scarce [4]. Reference standards for metabolites are often missing and the expenses for their acquisition or synthesis can be a limiting factor – especially in cases when several metabolites are formed from a single parent compound and the individual role of the metabolites in the environment (i.e., their relative distribution and stability) is unclear. Therefore, potential compounds need to be carefully prioritized.

Proton pump inhibitors (PPI) are among the most widely used pharmaceuticals. At present, PPI are considered the most effective therapy of gastric reflux and other acid-related disorders [5]. Pantoprazole (PPZ) is a top-selling PPI worldwide and is by far the most commonly used PPI in Germany. In fact, PPZ was ranked 2nd in the list of the most prescribed pharmaceuticals in Germany in 2019 [6]. Furthermore, in many countries, PPZ is a so-called over-the-counter (OTC) drug and can also be purchased without prescription. Despite the large consumption volumes, concentrations of PPZ in urban waters are comparatively low. Reported maximum concentrations of PPZ in wastewater and surface water are 180 ng/L [7] and 120 ng/L [8], respectively.

About 80% of an oral or intravenous dose of PPZ is excreted as metabolites in urine; the remainder is present in feces [9]. Metabolization of PPZ comprises a combination of phase I and phase II metabolism: Mainly oxidation/reduction of the sulfanyl group as well as 4’-O-demethylation and sulfatation/glucuronidation of PPZ and its reduced/oxidized forms were reported [10, 11]. Fluorinated pharmaceuticals such as PPZ, its metabolites and transformation products are of potential environmental stability and can accumulate in the environment [12].

Well as the elimination/transformation potential of the parent compound were screened for the presence of metabolites (Fig. 1) to identify the most promising PPZ related compound 4’-O-demethyl-PPZ-sulfide (M1), and two other reported metabolites included to a comprehensive and quantitative monitoring program. Surface water (aqueous phase and suspended particulate matter (SPM; n=11) of the Rhine at Koblenz (approx. 2 km upstream of the confluence of the Moselle River), were retrieved from the archive of the German Environmental Specimen Bank. The samples, which covered the period from 2005 to 2015, were collected at monthly intervals with sediment traps, were combined to annual samples and stored in liquid nitrogen. Raw water used for drinking water production and finished drinking water were sampled at four drinking water plants (DWTP) in Germany from May 2018 to November 2019. Water samples were also collected at nine bank filtration sites adjacent to the river Rhine and at one site adjacent the river Sieg on up to eight occasions between July 2020 and January 2021.

Toxicology and PBTK

P-166 Non-consideration of Pharmacokinetics Leads to Substantial Underestimation of the Environmental Exposure Associated with the Application of Pantoprazole
Laboratory experiment: In a laboratory batch test, the effectiveness of powdered activated carbon (PAC; NORIT®SAE SUPER) as a removal option for M1 in advanced wastewater treatment was assessed. Effluent from the final clarifier of the WWTP was used. A sample was taken to determine the initial concentration of the target analytes before the PAC treatment. Sample aliquots were filtered, transferred to amber glass bottles and spiked with different concentrations of dried PAC (5 mg/L; 10 mg/L; 20 mg/L). After 1 h and 24 h in an overhead shaker, the PAC of each sample was removed and the residual concentrations of the target compounds were determined. Liquid chromatography electrospray tandem mass spectrometry (LC-ESI-MS/MS) was applied for the quantification of studied compounds in water and SPM samples.

Table 1: PPZ and PPZ-metabolites used as analytes in the presented study.

<table>
<thead>
<tr>
<th>Name</th>
<th>Structure</th>
<th>CAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pantoprazole (PPZ)</td>
<td><img src="structure1.png" alt="Structure" /></td>
<td>102625-70-7</td>
</tr>
<tr>
<td>4’-O-demethyl-PPZ sulfide</td>
<td><img src="structure2.png" alt="Structure" /></td>
<td>141854-21-9</td>
</tr>
<tr>
<td>PPZ sulfide (M2)</td>
<td><img src="structure3.png" alt="Structure" /></td>
<td>102625-64-9</td>
</tr>
<tr>
<td>PPZ sulfone (M3)</td>
<td><img src="structure4.png" alt="Structure" /></td>
<td>127780-16-9</td>
</tr>
</tbody>
</table>

3. Results:
Daily average concentrations of PPZ in the WWTP influent and effluent ranged from 16 ng/L to 82 ng/L and from 36 ng/L to 96 ng/L, respectively. The average removal rates of M1 during the first and second sampling event were 33% and 12%, respectively. Influent and effluent concentrations of M2 were always in the low ng/L-range. M3 was only detected in some of the effluent and influent samples. In studied surface waters in Lower Saxony, concentrations ranged from <1.6 ng/L to 60 ng/L and from <1.1 ng/L to 1400 ng/L for PPZ and M1, respectively. Despite the large differences in their surface water concentration levels, concentrations of PPZ and M1 were highly correlated with each other (r=0.95; p<0.001). Figure 1 depicts the concentrations of M1 and CBZ along the studied stretch of the river Rhine from Basel to Duisburg and of three Rhine tributaries.

![Figure 1](image1.png)

Figure 2: Concentrations of M1 in SPM collected from the river Rhine at Koblenz (green points) and annual prescription volumes of PPZ in Germany (dotted line) from 2005 to 2015. SPM analyses were performed in triplicate. The solid line is the smooth line from a fitted generalized additive model (GAM). The shaded area corresponds to the pointwise 95% confidence interval for the GAM smooth terms.

![Figure 2](image2.png)

Figure 3: Changes in the relative concentration in % of studied compounds and contact times of 1 h (left) and 24 h (right) for different PAC dosages. Experiments were conducted with effluent from the final clarifier of a WWTP.

![Figure 3](image3.png)
Figure 2 shows the mean annual concentrations of M1 bound to SPM collected from the river Rhine at Koblenz and the annual prescription volume of PPZ in Germany for the time period 2005 to 2015. The concentrations of M1 in SPM have strongly increased from 0.53±0.09 ng/g dry weight in 2005 to 3.4±0.09 ng/g dry weight in 2015.

The results from the lab scale PAC adsorption experiments of studied compounds are depicted in Figure 3. After a contact time of 1 h and a PAC dosage of 20 mg/L, M1 (C0: 2000 ng/L) and PPZ (C0: 120 ng/L) were removed by more than 97%.

Table 2 list the results from the sampling at four drinking water treatment plants (DWTPs). While M1 was detected in all raw water samples at concentrations up to 250 ng/L, it was absent (i.e., <1.1 ng/L) in the corresponding finished drinking waters.

<table>
<thead>
<tr>
<th>DWTP</th>
<th>raw water</th>
<th>treatment</th>
<th>finished water</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>250</td>
<td>Filt.→O2→Floc.→ArRe→O3→Floc.→Filt.→GAC→DeAc→UV</td>
<td>&lt;1.1</td>
</tr>
<tr>
<td>2</td>
<td>87</td>
<td>Bank filtr.→O2→Filt.→DeAc</td>
<td>&lt;1.1</td>
</tr>
<tr>
<td>3</td>
<td>110</td>
<td>Bank filtr.→O2→Filt.→DeAc</td>
<td>&lt;1.1</td>
</tr>
<tr>
<td>4</td>
<td>42</td>
<td>Floc.→DeAc→O2→Floc.→Filt.→GAC→UV→ClO2</td>
<td>&lt;1.1</td>
</tr>
</tbody>
</table>

**Table 2:** Concentrations of M1 in ng/L in raw water (i.e. surface water) and finished drinking water of four drinking water treatment plants using different treatment options. Filt.: filtration (multi-layer filter or sand filtration); O2: aeration; Floc.: flocculation; ArRe: artificial groundwater recharge; O3: ozonation; AC: granular activated carbon; DeAc: deacidification; UV: UV disinfection; ClO2: chlorination.
4. Discussion:
During both study periods, the average effluent concentration of PPZ was approx. 20% higher than the average influent concentration. Negative removal for PPZ, which was also observed in an ultrafiltration membrane bioreactor experiment by Mousel et al. [15], could indicate the transformation of PPZ metabolites (e.g. via enzymatic cleavage of glucuronides and other conjugated metabolites) and subsequent release of the parent compound during wastewater treatment. The by far highest effluent concentrations of studied PPZ metabolites were found for M1 (mean effluent concentration: 990 ng/L). In fact, effluent concentrations of M1 were about 15 times higher than compared to PPZ.

The average concentration of M1 (190 ng/L) in studied surface waters in Lower Saxony exceeded that of the parent compound PPZ (6.4 ng/L) by a factor of approx. 30. This ratio is about two times higher than the ratio determined for WWTP effluents and could suggest that M1 is more persistent in surface waters than the parent compound. The strong positive linear correlation between M1 and CBZ indicates that the predominant source of M1 in surface waters is municipal WWTP effluent, which is to be expected for a urinary metabolite.

When looking at the concentrations of M1 and CBZ in the Rhine a general increase in the in downstream direction becomes apparent, which already has been observed for other wastewater-borne contaminants such as sulfamic acid [14], and which can be explained by the increase in the effluent fraction of the river from the Upper Rhine to the Lower Rhine. Due to the absence of experimentally obtained ecotoxicology data for the metabolites of PPZ, freshwater predicted no effect concentrations (PNECs) were derived using the quantitative structure–toxicity relationship (QSTR) model [16] of the toxicity prediction program ToxTrAMs. The provisional PNECs of the studied metabolites were all lower than that of the parent compound PPZ (PPZ: 28 µg/L, M1: 4.7 µg/L, M2: 6.1 µg/L, M3: 18 µg/L). It is plausible that PPZ is less toxic than some of its metabolites, since PPZ, being a pro-drug, is designed to be pharmacologically inactive. The highest surface water concentration of M1 found in the present study was about 3–4 times lower than its provisional PNEC.

The analysis of archived SPM samples from the Rhine at Koblenz showed that the SPM concentration of M1 in the Rhine at Koblenz was highly positively correlated (r=0.91; p<0.001) with the prescription volume of the parent compound PPZ (Figure 2). Note that the data only includes the prescriptions for people with statutory health insurance (approx. 90% of the total population in Germany). Furthermore, in 2009, PPZ was released from its prescription-only status in Germany. It can be assumed, that the actual usage of PPZ in Germany, especially after the year 2009, was considerably higher than what is reflected by the prescription volumes.

The sampling at DWTPs suggests that M1 can be sufficiently removed from contaminated source waters by commonly applied purification processes (multi-layer filter/sand filtration; ozonation; granular activated carbon; UV disinfection; chlorination) even though the data does not provide information on the efficiency of the individual treatment steps (Table 2). PPZ was only found in the source water samples of two DWTP (up to 2.0 ng/L). At studied bank filtration sites, the dilution-corrected concentrations of M1 decreased considerably during aquifer passage. In fact, at six sampling sites, the average removal of M1 was ≥80%. No general tendency towards higher or lower removal at different redox conditions was apparent.

Lab-scale adsorption on activated carbon experiments proved that this technique can efficiently remove M1 and PPZ from wastewater. In fact, adsorption was even higher than for CBZ (C0: 470 ng/L) (Figure 3), a compound with an elevated affinity for adsorption onto PAC [17].

5. Conclusions:
PPZ is among the most widely used pharmaceuticals worldwide. Despite its high consumption, PPZ concentrations in environmental water samples reported in the literature are comparatively low. This is due to extensive metabolization of PPZ in the human body and non-consideration of PPZ metabolites in previous monitoring studies. Using HRMS analyses, M1 was identified as the most relevant PPZ-related compound for environmental studies. M1 was found to be ubiquitously present in WWTP influents and effluents, as well as in wastewater-impacted water bodies in Germany. The parent compound PPZ as well as two other known metabolites of PPZ were detected at much lower concentrations.

While post-treatment with activated carbon can substantially reduce the release of M1 to the aquatic environment, additional studies are needed to elucidate and assess the main TPs of M1 during the ozonation of wastewaters. The presented study shows that information on the absorption, distribution, metabolism, and excretion (ADME) of medicinal products after administration must be taken into account in comprehensive monitoring programs in order to adequately describe their environmental occurrence and relevance.
Toxicology and PBTK

P-162  Epigenetic transgenerational effects of maternal exposure to low-dose DEHP on sperm functions and DNA methylation in male offspring rats

6. Acknowledgments:
This study was financially supported by the Lower Saxony Water Management, Coastal Defence, and Nature Conservation Agency (NLWKN) and the Environmental Ministry of Lower Saxony under grant no. 54711/1552-72/2018-2.5. Furthermore, we thank the German Environmental Specimen Bank for providing SPM samples. We would also like to acknowledge the anonymized DWTP and the WWTP for allowing us to collect samples. We are thankful to Reza Aalizadeh for providing the ecotoxicological data.

7. References:
Introduction: Numerous studies have demonstrated that non-alcoholic fatty liver disease (NAFLD) can have significant neurological implications, including anxiety, depression, and cognitive disorders. Moreover, exposure to environmental pollutants has been identified as a contributing factor in the development of this liver pathology, commonly referred to as toxicant-associated steatohepatitis (TASH). Importantly, these environmental pollutants can exacerbate both liver and brain-related consequences. The objective of this study is to establish a model that can effectively investigate the cerebral consequences of hepatic steatosis when exposed to a mixture of polycyclic aromatic hydrocarbons (PAHs). The model will allow for the examination of the interactions between hepatic steatosis and PAH exposure and their impact on the brain, specifically focusing on the effects of a high lipid diet and co-exposure to a mixture of polycyclic aromatic hydrocarbons (PAHs).

Materials and Methods: To induce moderate hepatic steatosis without associated obesity, a preliminary experiment was conducted to define the accurate duration of the enriched diet. The rats were therefore subjected to a high-fat and high-cholesterol diet (HFHC) for 30 to 90 days. Additionally, the rats were exposed to a mixture of 18 PAHs three times a week at a dose of 0.8 mg/kg. The study therefore involved four groups as followed: control, PAH, HFHC, and HFHC+PAH. The liver state was first assessed on 6 rats per groups at 30, 60 and 90 days through histological analysis of tissue morphology, biochemical measurements of liver enzyme activities in plasma and a glucose tolerance test (GTT). Furthermore, a battery of behavioural tests related to activity, anxiety, depression, memory, and stress response was conducted on 9 additional rats per groups from day 70 to 90.

Discussion and Conclusion: Histological examination of the liver in the NAFLD group revealed a progressive development of steatosis, characterized by an increase in lipid droplet deposition in hepatocytes, greater numbers of ballooned hepatocytes and cellular inflammation (higher inflammatory foci/field) after 90 days of treatments.

These histological lesions were associated with a significant increase in liver enzyme activities since ASAT (193.7 U/L vs 336.8 U/L) and ALAT (75.0 U/L vs 193.3 U/L) levels were increased by a factor of 1.7 and 2.7, respectively, in the NAFLD group compared with the control group. A significant increase in plasma cholesterol (+62%) and decreases in HDL cholesterol (-71%) and triglyceride (-54%) levels were observed in the NAFLD group compared to the control group. In the HFHC group co-exposed to PAHs, hepatocyte ballooning and an inflammatory state were enhanced compared with the HFHC group. The GTT results indicated no significant variations in blood glucose levels, suggesting that these hepatic lesions were not insulin-dependent.

Interestingly, PAH co-exposure appeared to limit the increase in blood glucose levels following 60 minutes of restraint stress, indicating potential impairment of the hypothalamic-pituitary-adrenal axis (HPA). Analysis of plasma and hair cortisol levels by LC-MS/MS is currently underway to further investigate this finding.

At the 90-day mark, the results pertaining to PAH co-exposure show promising outcomes, as PAHs were found to increase hepatocyte damage and inflammation. The ongoing behavioural evaluation will provide more information regarding the neurological consequences of this dual exposure at the central level.

Acknowledgments: The authors would like to express their gratitude to the Foundation for Medical Research for generously funding this project (ENV202109013686).
P-168  Pharmacokinetic study of valproic acid in the context of co-exposure to a-HBCDD, a brominated flame retardant of high concern, to assess the autism spectrum disorders in rats

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Introduction: Assessement to brominated flame retardant exposure, including that of ∼-hexabromocyclododecane (∼-HBCDD), on the etiopathogenesis of autism spectrum disorders (ASD), is an important environmental issue. Indeed, ∼-HBCDD is classified as a very high concern substance based on the potential adverse effects for human health, particularly developmental neurotoxicity. The aim of this study was to investigate the pharmacokinetics (PK) of valproic acid (VPA) in the context of co-exposure to a-HBCDD in rat, especially during gestation. Before evaluating the role of ∼-HBCDD as a susceptibility factor for ASD using this model, it was essential to understand the pharmacokinetic interactions between VPA and -HBCDD.

Materials and Methods: Female Wistar rats were subjected to a repetitive oral dose of ∼-HBCDD (100 ng/kg/day in oil) for 12 days. This was followed by a single intraperitoneal dose of VPA (500 mg/kg body weight) on GD 12 of gestation (GD12) or a daily oral dose of VPA (500 mg/kg body weight/day) for 3 consecutive days (from GD 10 up to 13). This study evaluated the effects of co-exposure in pregnant and non-pregnant animals to understand potential changes in the pharmacokinetics of VPA and -HBCDD under these physiological conditions. Sequential blood samples were collected from 20 minutes to 18 hours post-exposure to VPA. Concentration levels of valproic acid and -HBCDD in serum were determined using LC-MSMS. PK parameters were calculated using a non-compartmental model and The PK Solutions™ software package (version 2.0.6).

Discussion and Conclusion: The PK modelling indicates that exposure to -HBCDD did not significantly affect the pharmacokinetics of VPA in pregnant or non pregnant rats. However, the administration of VPA affected the pharmacokinetics of -HBCDD. Similar Cmax values were observed for -HBCDD alone or for -HBCDD-VPA in rats exposed in IP, whereas a 3-fold increase in the -HBCDD Cmax was showed in rats orally exposed to VPA. The VPA altered the metabolism of -HBCDD, particularly through the influence of CYP450 enzymes (a significant increase in gene expression of Cyp3a1 (p<0.01) and decrease in gene expression of Cyp2c11 (p<0.001), resulting in increased circulating blood concentrations of -HBCDD when administered orally. In addition, the administration of VPA at a dose of 500 mg/kg resulted in foetal toxicity (significant decreases were observed in both embryo (-43%, p<0.001) and placenta weight (-54%, p<0.001)) and lethality, with greater severity observed with the oral route than with the intraperitoneal route. In contrast, -HBCDD did not aggravate VPA-induced intrauterine growth retardation or embryonic lethality by any route of exposure. This study points out a potential drug-pollutant interaction, suggesting how the drug can alter the pharmacokinetics of a pollutant which result in a significant increase of internal exposure levels. These findings highlight the importance of assessing the neurotoxic risks associated to co-exposures of pollutants, particularly for vulnerable populations such as pregnant women and infants in the first months of life.

Acknowledgments: The authors would like to express their gratitude to A2F young research grant (2019-2020) - Lorraine University France, Lorraine University excellence Mobility DrEAM episode 7 (2021-2022).