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PFASs: What can we learn from the European Human Biomonitoring Initiative HBM4EU

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ABSTRACT

Per- and polyfluoroalkyl substances (PFASs) were one of the priority substance groups selected which have been investigated under the ambitious European Joint programme HBM4EU (2017–2022). In order to answer policy relevant questions concerning exposure and health effects of PFASs in Europe several activities were developed under HBM4EU namely i) synthesis of HBM data generated in Europe prior to HBM4EU by developing new platforms, ii) development of a Quality Assurance/Quality Control Program covering 12 biomarkers of PFASs, iii) aligned and harmonized human biomonitoring studies of PFASs. In addition, some cohort studies (on mother-child exposure, occupational exposure to hexavalent chromium) were initiated, and literature researches on risk assessment of mixtures of PFAS, health effects and effect biomarkers were performed. The HBM4EU Aligned Studies have generated internal exposure reference levels for 12 PFASs in 1957 European teenagers aged 12–18 years. The results showed that serum levels of 14.3% of the teenagers exceeded 6.9 μg/L PFASs, which corresponds to the EFSA guideline value for a tolerable weekly intake (TWI) of 4.4 ng/kg for some of the investigated PFASs (PFOA, PFOS, PFNA and PFHxS). In Northern and Western Europe, 24% of teenagers exceeded this level. The most relevant sources of exposure identified were drinking water and some foods (fish, eggs, offal and locally produced foods). HBM4EU occupational studies also revealed very high levels of PFASs exposure in workers (P95: 192 μg/L in chrome plating facilities), highlighting the importance of monitoring PFASs exposure in specific workplaces. In addition, environmental contaminated hotspots causing high exposure to the population were identified.

In conclusion, the frequent and high PFASs exposure evidenced by HBM4EU strongly suggests the need to take all possible measures to prevent further contamination of the European population, in addition to adopting

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remediation measures in hotspot areas, to protect human health and the environment. HBM4EU findings also support the restriction of the whole group of PFASs. Further, research and definition for additional toxicological dose-effect relationship values for more PFASs compounds is needed.

1. Introduction

Per- and polyfluoroalkyl substances (PFASs) were one of the priority substance groups selected in the European Human Biomonitoring Initiative HBM4EU. Several policy-questions on exposure and health effects were developed and addressed under the Scoping Document at the beginning of the research program, including an attempt to answer whether existing regulations on PFASs were sufficient to protect human health. These policy questions were addressed in the Scoping Document for PFASs[1] at the beginning of the research program.

The HBM4EU project period coincided with growing awareness of the potentially harmful effects of PFAS exposures in the general population. As an example, in 2020 the European Food Safety Authority derived a new tolerable weekly intake (TWI) of 4.4 ng/kg bw per week, for the sum of PFOA, PFNA, PFHxS and PFOS (EFSA, 2020). The new TWI was derived on the basis of effects on the immune system, more specifically the reduction of antibody response after vaccination in children (Abraham et al., 2020). This TWI corresponds to a blood serum level of 6.9 μg/L in women of childbearing age, which would prevent the breastfed infant from exceeding 17.5 μg/L in serum; the reference point derived by EFSA. In this approach, EFSA assumed equal potency for the four substances as no studies were available to derive relative potency factors (RPFs) for human effects. The dietary exposure assessment performed by EFSA showed that large numbers of the EU-population exceeds the new TWI.

While most individuals are exposed to background-levels of PFASs, some populations living near contaminated sites are exposed at moderate, high or extremely high levels (Xu et al., 2020, 2021; Ingelido et al., 2018). The main sources of PFASs contamination at hotspots are (historical) PFASs production, which contaminated the soil and groundwater, or the use of PFASs containing products such as PFASs containing firefighting foams or PFASs contaminated biosolids applied as soil improving materials. The list of suspected PFASs contaminated sites in the EU is continuously growing. Occupational exposure to PFASs has only been investigated in a few studies (Olsen et al., 2007), such as in fluoropolymer manufacturing, firefighters when using aqueous film-forming foams (AFFF) or professional ski waxers (Langenbach and Wilson, 2021). However, PFASs have a wide range of applications; therefore, occupational exposure is expected to occur in other sectors as well (Langenbach and Wilson, 2021).

This publication synthesizes HBM4EU's activities, results and knowledge gained within the five years and half of HBM4EU and highlights how they may be used to inform science-based policy decisions on PFASs in Europe.

2. Results and discussion

2.1. Exposure to PFASs

2.1.1. General population: The HBM4EU teenagers aligned study

One of the overall objectives of HBM4EU was to assess exposure to PFASs among other priority chemicals in Europe in the best possible harmonized way.

A prioritized list of biomarkers, matrices and analytical methods was developed for a broad spectrum of PFASs, including both well-known PFASs (e.g. perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkane sulfonic acids (PFSAs) as well as novel PFASs (e.g. HFPO-DA (GenX) and ADONA) which were used as alternatives to the legacy ones (Vorkamp et al., 2021). Generally, the preferred matrix for targeted PFASs human biomonitoring is blood, specifically serum or plasma, but breast milk and urine may also be desired matrices in specific cases.

The HBM4EU QA/QC program covered analysis of 12 PFASs in serum. The laboratory proficiency tests included four rounds in collaboration with reference laboratories worldwide (Esteban López et al., 2021). Of the total of 26 eligible candidate laboratories that had initially been invited to participate in the HBM4EU QA/QC program for PFASs, 21 laboratories from 12 European countries qualified for the analysis of at least six PFASs and seven laboratories covered the selected 12 PFASs. This approach was followed within the HBM4EU aligned studies (Gilles et al., 2021, 2022; Govarts et al., 2023), in which exposure data to 12 PFASs in serum from 1957 teenagers aged 12–18 years, sampled between 2014 and 2021 in nine European countries geographically distributed across Europe, were obtained. The description of the approach of the HBM4EU aligned studies has been described elsewhere (Gilles et al., 2021, 2022; Govarts et al., 2023). These current serum PFASs measurement data indicate a geographical difference in exposure, with higher concentrations in Northern and Western Europe for the legacy PFASs (PFOA, PFNA, PFHxS, PFOS) (Fig. 1). Between 1 and 24% of the subjects across the nine data collections have levels above the serum level corresponding to the TWI for the sum of PFOA, PFNA, PFHxS and PFOS of 6.9 μg/L (EFSA, 2020), with an overall exceedance of 14%. For the PFASs which were detected at lower levels (PFPeA, PFHxA, PFHpA, PFDA, PFUnDA, PFDoDA, PFBS and PFHpS), the detection frequencies observed were strongly dependent on the limit of quantification (LOQ) reached in the laboratories, indicating it is crucial to lower LOQs for further interpretation of the data.

Higher serum levels of PFNA and PFOS were associated with higher consumption of fish and seafood and higher consumption of eggs (increase in serum levels by 20 and 21% for fish and seafood and by 14 and 11% for eggs respectively). Furthermore, higher PFASs was linked to higher consumption of offal (increase in serum levels by 14%) and local food (increase by 40%). (Richterová et al., 2022). Fig. 1 depicts the HBM4EU exposure indicator and represents the median (P50) serum concentrations in European teenagers.

2.1.2. Exposure in hotspots

In the framework of HBM4EU, a network of experts focusing on hotspots was established that calls for systematic identification of hotspots in the EU and the establishment of human biomonitoring studies aligned on these hotspots. This would offer the opportunity to obtain larger and more comparable datasets to better investigate the relationship between exposure levels and health effects. The expert HBM4EU hotspots group also developed a short guidance document on performing HBM at hotspots and how to communicate results and potential risks and also addressed the need to identify hotspots systematically (Brouwere et al., 2022).

2.1.3. Occupational exposure

Occupational exposure to PFASs may occur in the metal sector, especially electroplating activities. PFASs have been used as mist suppressants especially in chrome plating baths to prevent the evaporation of chromium (VI) vapours (Blepp et al., 2017; Glüge et al., 2020). PFOS was earlier the most important PFAS used in plating activities. Due to the restrictions of its manufacture and use, it has been largely replaced in the EU (EC, 2020).

[1] https://www.hbm4eu.eu/wp-content/uploads/2017/04/Scoping-document-on-per-and-poly-fluoralkyl-substances.pdf.

In HBM4EU an occupational study was conducted with the aim to study exposure to hexavalent chromium in various sectors (Santonen et al., 2019, 2022). This study also included biomonitoring of exposure to PFASs in a subset of workers performing chrome plating activities and some workers performing welding activities. Some of the chrome platers showed clearly elevated PFOS serum levels with the 95th percentile for PFOS among platers being 192 μg/L, which can be explained by the former application of PFOS in electroplating baths (Santonen et al., 2023). (Glüge et al., 2020) recently performed an analysis of the uses of PFASs, demonstrating that they are used in almost all industries. Considering this, it is highly likely that there are still various unrecognized sources of occupational exposure to PFASs which need to be identified, and their impact on workers' health needs to be assessed as well as exposure and risk reduction measures to be implemented.

2.2. Health effects of PFASs

2.2.1. General population: epidemiological studies

One of the first relatively large studies addressing health effects of PFASs in the general population examined the associations between concentrations of PFOS and PFOA in pregnant women with birth weight in their offspring (Apelberg et al., 2007; Fei et al., 2007). Both studies reported higher serum concentrations to be inversely associated with birth weight. Later studies from other birth cohorts largely supported these findings (Bach et al., 2014; Gao et al., 2021). Despite consistency of those reports, it has been suggested that they may reflect interindividual differences in uptake and excretion (Savitz, 2007), rather than true causal association. Very few studies have addressed the possible mechanism for lower birth weight with higher PFASs exposure. (Nielsen et al., 2020; Verner et al., 2015).

To identify mechanisms that could explain the inverse association between prenatal PFASs exposure and birth weight, a comprehensive review of existing data was conducted as part of HBM4EU (Gundacker et al., 2022). The results suggest that the thyroid-damaging effects of PFASs and their ROS-induced effects on adipocyte differentiation are possible mechanisms for body weight reduction. Other potential mechanisms related to decreased placental weight (Fei et al., 2008) include PFOS-induced detrimental effects on decidualisation (Yang et al., 2016), trophoblast cell viability and hormone release (Zhang et al., 2015), or angiogenesis (Forsthuber et al., 2022). The PFOS-induced inhibition of signaling through the vascular endothelial growth factor receptor 2 (VEGFR2) detected in the latter study suggests a mode of action related to an existing Adverse Outcome Pathway (AOP) addressing the adverse outcome "decreased birth weight". There is also some evidence that elevated PFOS concentrations are associated with decreased levels of insulin-like growth factor 1 (IGF1) in infant serum (Lopez-Espinosa et al., 2016) and in mouse liver and testis (Wan et al., 2011), and that PFOA decreases IGF2 methylation (Kobayashi et al., 2017). Growth factors may indeed play a central role, as decreased maternal IGF1 is associated with decreased fetal growth in humans and animals (Dimasuay et al., 2016).

Epidemiological studies have also shown relatively consistent findings between long-chain PFASs and increasing levels of serum cholesterol in both cross-sectional and prospective designs conducted in background and occupationally exposed subjects, which has been discussed in a HBM4EU review paper (Fragki et al., 2021). The molecular mechanism responsible for PFASs causing an increase in cholesterol levels is not fully understood, but may involve lipid uptake mechanisms including the bile acid surfactant system (Sinisalu et al., 2021), vesicle size, permeability of membranes or links to the immune system (Wit et al., 2022) as well as effects on PPAR receptors. Ecological comparison between people exposed to contaminated versus non-contaminated drinking water have also shown on average higher lipid concentrations, supporting a causal association (Li et al., 2020). The possible mechanism behind this association have also been addressed in the aforementioned HBM4EU publication (Fragki et al., 2021).

One of the outcomes that is currently considered more certain with respect to causality, is the effect of long-chain PFASs on the immune system, more specifically on antibody response following vaccination (Schrenk et al., 2020).

On the other hand, one weakness of reduced antibody response as the basis for setting a health-based guidance value (Schrenk et al., 2020) is that a link to more clinically adverse outcomes such as increased propensity of infections is not as well established in humans. Many published studies rely on retrospective parental reporting on their child's previous infections that can be considered at best as uncertain (Abraham et al., 2020; Granum et al., 2013). The two other studies relying on more accurate continuous prenatal reports (Fei et al., 2010) or registry-based outcomes for hospital admissions for infections in early childhood (Dalsager et al., 2016) have reported positive or no association, respectively. Lack of data using objective measures on propensity of infection was partly addressed within HBM4EU. One of those studies examined associations between pregnancy exposure to PFASs in relation to hospital admissions due to infections in children up to 4 years of age (Dalsager et al., 2021). That study, including ~1500 mother child pairs, found a relatively clear positive association between pregnancy concentrations of PFASs with higher propensity for hospital admission to infections in the offspring. Findings support the existing health-based guidance value for long-chain PFASs suggesting that reduced antibody titers may translate into increased propensity of infection, an outcome which is more clearly anchored in adversity compared to isolated observation on vaccination response.

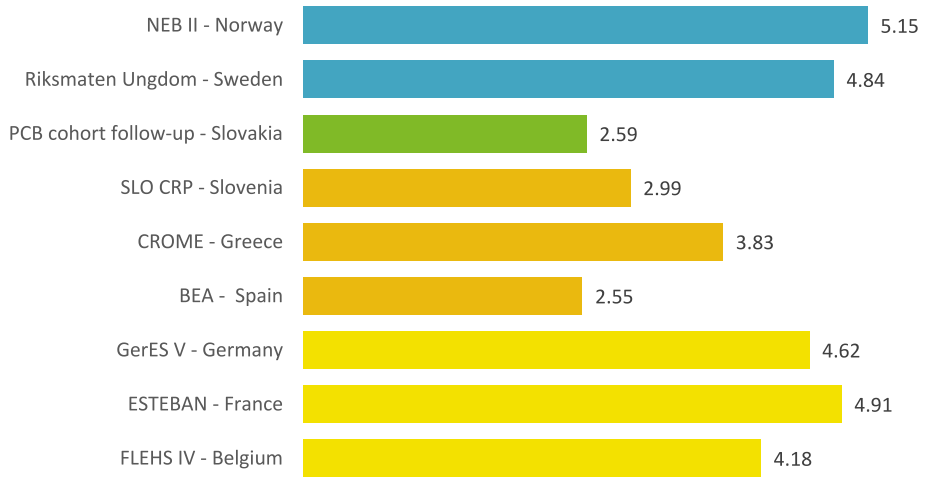

Fig. 1. Geographical differences in exposure to sum of PFOS, PFHxS, PFOA and PFNA in plasma/serum (μg/L) of teenagers in Europe by median value (P50).

The PFASs-induced pathways of immunotoxicity was also summarized and discussed in a review paper performed within HBM4EU, which strengthens the evidence that PFASs affect multiple aspects of the immune system and supports the overall conclusion that not only PFOA and PFOS, but also other members of the PFASs family alter immune functions in humans (Ehrlich et al., 2023).

2.2.2. Health effects in hotspots

HBM4EU has also conducted a review of the health effects of PFASs in hotspot regions. Health risks of PFASs have been studied in large populations which have suffered long term exposure to PFASs via contaminated drinking water. This has been facilitated as associations between groups with substantial exposure contrast offer the possibility of good statistical power to detect effects.

Although no HBM studies in hotspots have been performed within HBM4EU, the review on health effects in hotspot areas (Fletcher, 2022) has shown that hotspot studies enable identification of diseases associated with PFASs at clearly contrasting and relatively high exposures and body burdens. When the HBM4EU project was initiated very few hotspots had been identified in Europe, but this number increased considerably through the HBM4EU project period. There is an urgent need to map potentially PFASs contaminated sites and perform targeted HBM studies in hotspot areas, in future research programs such as the partnership for the assessment of risks from chemicals (PARC). It can be built on the HBM4EU network, which has been described above.

2.3. Biomarkers of effect

One of the aims of HBM4EU was the selection and implementation of effect biomarkers at large scales in future HBM studies in a systematic and standardized way, in order to complement exposure data with mechanistically-based biomarkers of early adverse effects. This study summarizes the prioritized existing biomarkers of effect for PFASs. The selection was made based on relevant mechanistic and/or AOP information, as well as on human data and health outcomes (Fernandez et al., 2021; Mustieles et al., 2018). Fig. 2 provides a graphic scheme for biomarkers of effect relevant for PFASs.

2.3.1. Omic and epigenetic effect biomarkers

Some molecular markers associated with PFASs exposure and related to reproduction, immunotoxicity, obesity and metabolic disorders were identified. For example, alterations in certain gene transcripts levels [NR1H2 (LXRB), ABCG1 & NPC1] involved in cholesterol metabolism and transport (Fletcher et al., 2013), as well as changes in the expression of genes linked to immune function (CYTIL1, IL27) (Pennings et al., 2016). PFASs have been investigated for endocrine disrupting properties in a broad range of *in vitro* assays, finding associations with the expression of certain nuclear receptors (e.g. AR, PXR, PPAR) (Caserta et al., 2013a, 2013b; La Rocca et al., 2012, 2014, 2015). Some studies have also involved epigenetic markers (DNA methylation, histone modification, microRNA expression), and oxidative stress markers as key mediators of some adverse health outcomes derived from early PFASs exposure (Kim et al., 2021).

Although molecular markers may constitute *per se* "biomarkers of effect"; however, their implementation in HBM studies should still be taken with precaution, given the limited epidemiological data on the molecular effects of PFASs. Incorporation of available information from experimental studies could help to construct more robust mechanistic pathways of PFASs toxicity (Fragki et al., 2021).

2.3.2. Immune biomarkers

Among the main effects linked to PFASs exposure are suppression of the antibody response to vaccination (i. e., reduced immune response), as well as lower levels of proteomic markers of inflammation) (Chang et al., 2016; Pennings et al., 2016; Salihovic et al., 2020). Among the biomarkers selected in HBM4EU for immunological effects are circulating antibody levels, basophil count, absolute eosinophil count (AEC), eosinophil cationic protein (ECP) concentrations, lymphocyte

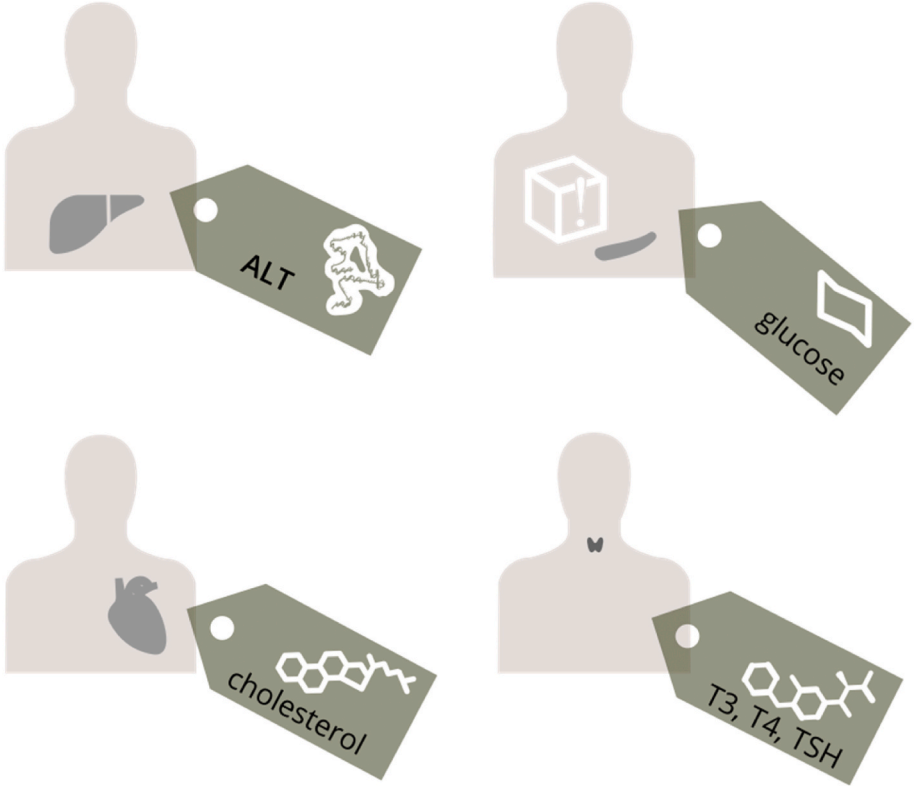

Fig. 2. Biomarkers of effect relevant for PFASs.

subpopulations [e.g. immunoglobulin (IG)E markers and specific IGE antibodies], as well as CC16 protein levels. However, further research is needed to better characterise the pathways of PFASs on the developing immune system.

2.3.3. Cardiometabolic biomarkers

Among cardiometabolic markers, alterations of lipid metabolism markers and increased total serum cholesterol levels have been identified as one of the most critical effects of PFASs on human health (EFSA, 2020). The information on other cardiometabolic markers is much less (Zare Jeddi et al., 2021). The implementation of biomarkers of chronic inflammation (proinflammatory adipokine, leptin, and proinflammatory cytokines, IL-6 and IL-1β biomarkers), in addition to clinical biomarkers of metabolic function (adiponectin, leptin, adiponectin/leptin ratio and HOMA-IR) could help to elucidate the role of PFASs in the development of metabolic diseases, together with anthropometric biomarkers and cardiometabolic risk scores (Li et al. , 2021a; Papadopoulou et al., 2021).

2.3.4. Biochemical effect biomarkers (reproductive and thyroid hormones)

2.3.4.1. Reproductive hormones. Maternal or offspring sex hormone levels have been used as biomarkers of reproductive effect in some epidemiological studies (Bach et al., 2014; Petersen et al., 2020; Itoh et al., 2016; Maisonet et al., 2015). Some cross-sectional studies have also described negative associations between serum PFOA, PFOS and PFUA concentrations and serum SHBG, FSH and testosterone levels (Tsai et al., 2017; Zhou et al., 2016), with more significant associations among males (Joensen et al., 2013), as well as with lower estradiol and progesterone production in nulliparous women (Barrett et al., 2015).

2.3.4.2. Thyroid hormones. PFASs also interfere with the signaling pathways of the thyroid hormones, with negative repercussions on pregnancy outcome and fetal-infant development (Ballesteros et al., 2017; Coperchini et al., 2021; Tsai et al., 2017). Currently, the onset of hypothyroidism in the population exposed to PFASs represents the most frequent thyroid effect of these pollutants. To assess normal thyroid function, triiodothyronine (T3), thyroxine (T4), FT3, FT4 and TSH levels should be taken into account. In this regard, findings suggest that PFASs are associated in an age- and sex-specific manner (Blake et al., 2018; Jain, 2013; Rickard et al., 2022), in addition to probably varying according to coexistence with other environmental pollutants (Shrestha et al., 2015).

2.4. Risk assessments based on combined exposure to multiple PFASs detected in the human serum of teenagers in the HBM4EU aligned study effort

In HBM4EU, different risk assessment approaches have been used to determine whether the population of European teenagers where PFASs levels were measured (Govarts et al., 2023) would be at risk of developing adverse health effects taking into account combined exposure to multiple PFASs. Three approaches were used for comparison: the TWI established by (EFSA, 2020), the hazard index (HI) approach, and the relative potency factor (RPF) approach. The HI and RPF approach were adapted following the EFSA approach to allow using HBM data as primary input. For the HI approach, four epidemiological studies of two critical health end-points indicated by EFSA, immunotoxicity (Grandjean et al., 2012; Kielsen et al., 2016) and birth weight decrease (Meng et al., 2018; Wang et al., 2016) were selected. The geometric mean or median PFASs serum concentrations was included in the HI approach as effect level (i.e. Point of Departure) when there was a statistically significant association between exposure to PFASs and the health outcomes. This resulted in inclusion of PFOA, PFHxS, and PFDA (Grandjean et al., 2012), PFOS, PFNA, PFDA, PFUnDA and PFDoDA (Kielsen et al., 2016), PFNA, PFDA, PFUnDA, and PFDoDA (Wang et al., 2016), and PFOS, PFOA, PFNA, and PFHpS (Meng et al., 2018) in the HI approach, respectively (Bil et al., 2023). For the RPF approach, toxicokinetic models were generated for 10 PFASs to estimate the internal exposure in the male rat at the blood serum level over time. These internal exposures were then used to derive internal RPFs based on liver effects in male rats (Bil et al., 2022). This resulted in inclusion of PFHxA, PFOA, PFNA, PFDoDA, PFBS, PFHxS and PFOS in the RPF approach, respectively (Bil et al., 2023).

Whenever the approach allowed for this, exposure to multiple PFASs was considered at the individual level in order to arrive at precise mixture exposure values. Summed PFASs serum concentrations were calculated per individual, prior to retrieving the 50th and 95th percentile of the serum concentration distribution per study cohort. Based on any of the approaches considered, an increased risk to adverse health effects as specified also by EFSA 2020, was calculated in the highly exposed part of the HBM4EU study population. Even though these approaches differed in the number of PFASs included, their underlying hazard data, and their assumptions used to arrive at the outcomes, all three assessments point in the same direction. Overall, the results are in line with the risk characterization of (Schrenk et al., 2020) and it is clearly showed that adverse health effects may arise due to PFASs mixture exposure in the European population (Bil et al., 2023). Fig. 3 Fig. 3 shows the proportion of European teenagers whose combined exposure to PFOA + PFNA + PFHxS + PFOS, is above the EFSA health-based guidance value of 6.9 μg/L.

3. Science to policy

A key focus of the HBM4EU project was to generate science which can support decision-making and risk management of chemicals.

Within a workshop in the frame of HBM4EU the development and use of HBM based indicators have been discussed in order to learn from and ensure interoperability with other European indicators (Buekers et al., 2018). Further, HBM based indicators have been developed within HBM4EU to track progress, they present HBM data in an easy understandable and comparable way and they can be included e.g. in state-of-the-environment reporting at EU- and national level. Continued investment in monitoring, ideally with a time interval of two to three years between data points, would be needed for this purpose. Thus, to support the use of HBM4EU results for policy making related to PFASs, two workshops were organized (April 2021 and March 2022). Invitees for the workshops were HBM4EU researchers, representatives of national HBM studies and national authorities, representatives from various DGs of the European Commission and EU agencies. The workshops were organized in a confidential setting, to make it possible to discuss results that were not yet publicly available. Workshop participants concluded that HBM research plays an important role in raising awareness and putting the subject on the political agenda. In that regard, HBM data have played an important role in the development of the European Commission's PFASs strategy. Furthermore, results of HBM4EU, e.g. on health effects, mode of action and mixture risk assessment will support the forthcoming REACH restriction proposal to cover a wide range of PFASs uses (HBM4EU, 2021). Given the fact that many new policy actions on PFASs are on the table, HBM4EU results can become an important baseline to follow up effectiveness of policy measures.

A PFASs policy brief was prepared in HBM4EU (2022), which summarized the key findings, to inform future policy actions.

HBM4EU data and results have been reported within the public consultation on the EFSA scientific opinion on the risk to human health related to the presence of perfluoroalkyl substances in food, and have been shared with the member states supporting the call for evidence for a broad PFASs restriction. Further *ad hoc* support the EU-processes, e.g. in the development of HBM-indicators to support the Chemicals strategy for sustainability, the Zero pollution and the 8th Environmental action

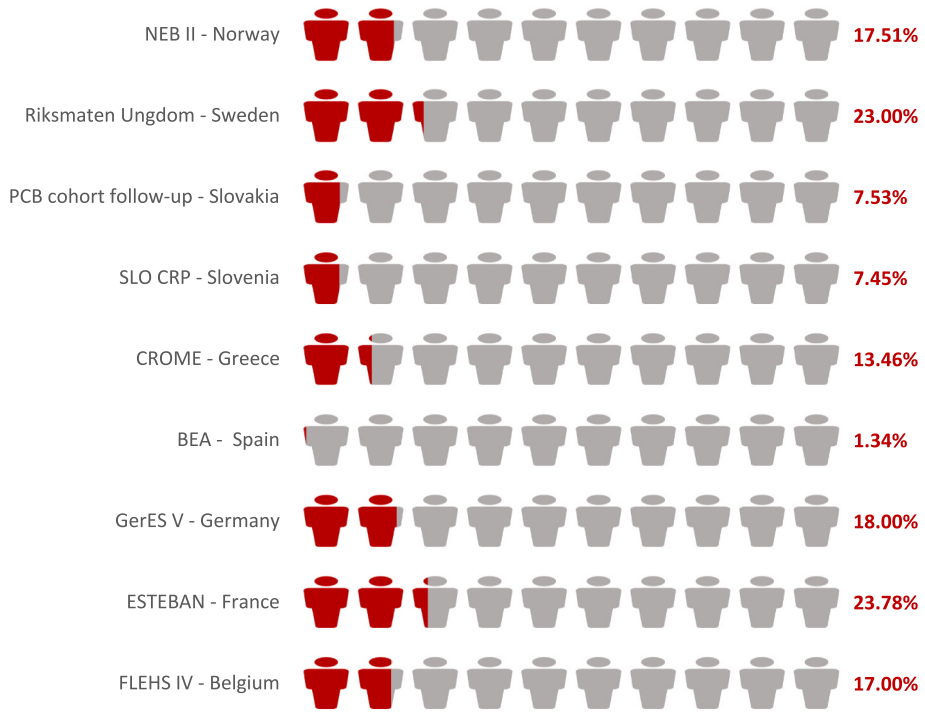

Fig. 3. Share of European teenagers with combined exposure levels to PFOA + PFNA + PFHxS + PFOS exceeding health-based guidance value of EFSA (6.9 μg/L).

programme indicators may be highly useful to track effectiveness of policies on PFASs (Vicente et al., 2023). HBM4EU will support countries facing PFASs contamination issues in hotspots, as a guidance has been developed and discussed with experts in the field.

The results of HBM4EU clearly support the need for far-reaching policy action on PFASs to reduce exposure for the general population, in PFASs hotspots and for occupationally exposed citizens.

4. Conclusions and recommendations

HBM4EU has implemented a broad range of activities and produced highly valuable results on PFASs exposure and health effects. A network of 21 highly qualified laboratories has been established ensuring that human biomonitoring of PFASs can be performed in a highly quality assured way in Europe. High quality human biomonitoring data have been generated for risk assessment. The large-scale aligned and harmonized studies under HBM4EU and research on health effects in HBM studies have revealed that the exposure of European teenagers exceeds health based guideline values, which is a concern for present and future generations. Exposures at specific occupational settings and in the increasingly emerging hotspots have been revealed to be even orders of magnitude higher and of utmost concern.

In order to prevent future pollution by PFASs, it is key to prevent further emissions and accumulation of PFASs in the environment and the use in products. The broad restriction of PFASs for all non-essential uses and activities on other levels are therefore critically needed. Cleaning up of existing hotspots is key to prevent human (and biotas) exposure from legacy uses of PFASs. Effective prediction of potential hot spot polluted areas will require mapping of sites where PFASs have been or currently are being produced, manufactured or used. It is recommended, that studies on PFASs hotspots also in the future will compile data from different cohorts to explore health effects. Further research is needed on the elimination of PFASs from the body, as well as a better understanding of the uptake, distribution (including into fatty tissues) and metabolism of PFASs precursors of PFAAs (Ng et al., 2021).

In following up from the aligned studies time trends will show the effectiveness of measures. Attention should be paid to the sampling scheme and accuracy in terms of harmonization, specifically on issues such as the representativeness of the population, inclusion of highly exposed and vulnerable citizens, including children (Trier et al., 2011). In order to take into account that the number of PFASs is constantly growing and, according to recent estimates, already covers millions of substances, it is necessary to further develop and harmonise methods that allow for quantifying and identifying emerging PFASs substances as well as the measurement of total PFASs content. In future HBM Studies also other PFASs compounds (as. For ex. FTOHs, etc.) should be integrated as also the research on additional toxicological doses-effect relationship values (TDI, Unit Risks, etc.).

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Glossary

AEC: absolute eosinophil count
ADONA: ammonium 4,8-dioxa-3H-perfluorononanoate
AFFFs: aqueous film-forming foams
AOP: adverse outcome pathway
AR: androgen receptor
C8: perfluorooctanoic acid
CIC: combustion ion chromatography
CC16: club cell protein 16
EC: European Commission
ECP: eosinophil cationic protein
EDC: endocrine disrupting chemical
EFSA: European Food Safety Authority
EOF: extractable organofluorine
FT3: free triiodothyronine
FT4: free thyroxine
HBM: human biomonitoring
HBM4EU: European Human Biomonitoring Initiative
HFPO-DA (GenX): hexafluoropropylene oxide-dimer acid
HI: hazard index
HOMA-IR: homeostatic model assessment for insulin resistence
IG: immunoglobulin
IGF: insulin-like growth factor
IL: interleukin
P50: 50th percentile
P95: 95th percentile
PARC: partnership for the assessment of risks from chemicals
PFAA(s): perfluoroalkyl acid(s)
PFASs: per- and polyfluoroalkyl substances
PFBS: perfluorobutanoic acid
PFCA(s): perfluoroalkyl carboxylic acid(s)
PFDA: perfluorodecanoic acid
PFDoDA: perfluorododecanoic acid
PFHpA: perfluoroheptanoic acid
PFHxA: perfluorohexanoic acid
PFHxS: perfluorohexane sulfonate
PFNA: perfluoro-n-nonanoic acid or perfluoro-n-nonane carboxylate
PFOA: perfluoro-n-octanoic acid or perfluorooctane carboxylate
PFOS: perfluoro-n-octane sulfonate or perfluorooctane sulfonic acid
PFOSA: perfluorooctane sulfonamide
PFPeA: perfluoropentane acid
PFUnDA: perfluoroundecanoic acid
PPARs: peroxisome proliferator-activated receptors
PTFE: polytetrafluoroethylene
PXR: pregnane X receptor
QA/QC: quality assurance/quality control
RPF: relative potency factor
T3: triiodothyronine
T4: thyroxine
TOPA: total oxidizable precursor assay
TSH: thyroid stimulating hormone
TWI: tolerable weekly intake
VEGVR: vascular endothelial growth factor receptor