Prioritised substance group: Per- and polyfluoroalkyl substances (PFASs)

Perfluorinated substances (PFAS)	Maria Uhl (EAA, AT), Þórhallur Ingi Halldórsson, Ingrid Hauzenberger, Christina Hartmann
Perfluorinated substances (PFAS)	Xenia Trier, Tony Fletcher, Philippe Grandjean, Marqués Bueno Montserrat, Wang Zhanjun

Short overview of results of the activities carried out within HBM4EU in 2020 to answer the policy questions with reference to corresponding deliverables.

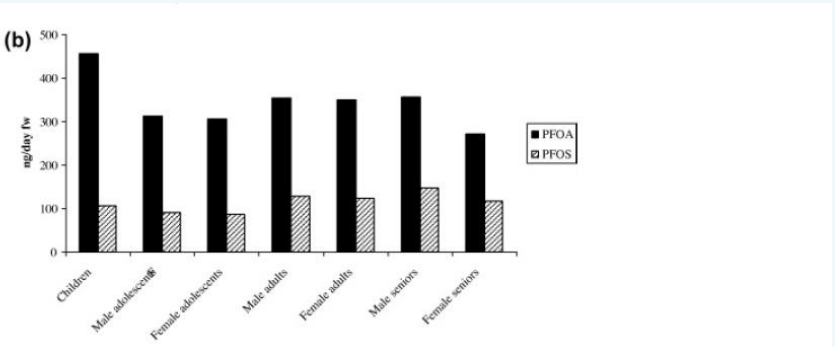
Policy Question	Short Summary of Results
1. What is the current exposure of the EU population to PFASs and do they exceed Guidance values (reference and HBM values), where available?	To examine the current exposure of the EU population and the respective health effects is task of several work packages and tasks within HBM4EU. WP8.1 Alignment of national studies contains the HBM4EU strategy with the overall aim to align ongoing and/or planned studies to collect data from HBM4EU priority substances with EU wide coverage. The current exposure will be assessed on the basis of samples collected between 2014 and 2019 related to three age groups. The strategy includes countries from different European regions focussing on children aged 6-11 years, adolescents aged 12-19 years and adults aged 20-39 years. In addition, an inventory on national HBM studies that could be part of the first HBM4EU Human Biomonitoring program was made. For PFAS, the proposed sampling scheme includes adolescents (12-19 years of age), and time trend analysis. For the investigation of PFAS exposure, samples were collected from different regions (and 11 countries): 484 samples from the North, 548 from the South, 900 from the East and 900 from the West. Thus, a total of 2,832 samples are available. In these samples 8 to 12 PFAS are measured including PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFBS, PFHxS, PFHpS, and PFOS (sum of all isomers). These data will provide a basis for exposure and risk assessment for a part of the European population, namely teenagers. Until now, four studies have completed the analysis of PFAS including studies from the countries Spain, Belgium, Sweden, Norway. The analysis of PFAS in the remaining cohorts from Slovenia, Greece and Slovakia are ongoing. Exposure data as well as existing data will be compared and evaluated, and will be integrated into IPCHEM. In addition, the new exposure data generated will be used to fill data gaps identified. Further, data will be generated to distinguish exposures of relevant population groups including sensitive sub-groups (e.g. adults vs children of different age groups, males vs females). In addition, within WP8.5, Targeted occupational studies with EU added values and coordination of activities occupational PFAS exposure is investigated together with chromate exposure. Therefore, plasma samples of workers are analysed from five studies including a total of 158 samples. The collection of occupational samples has already been completed. For the collection of new samples and data WP7.3 Questionnaires development basic questionnaires were developed which were further spitted into questionnaires for the single priority substances. With respect to PFAS, a basic questionnaire for adolescents aged 12-19 years was

Policy Question	Short Summary of Results
	developed including additional specific single questionnaires for PFAS. In D7.6, questionnaires were developed for PFAS exposure (specific and general questions). In WP 8.4, Targeted fieldwork in combining HES and HBM surveys influencing and interfering factors for sampling and storage were identified, whereas for each substance group recommendations were made to avoid sample contamination or inappropriate storage conditions that may influence sample quality and hence the outcome of the analysis. For PFAS, specific recommendations are given, e.g. avoiding Teflon and other fluropolymers as well as glass in the sampling material, and information on shipment and biobanking. In an additional deliverable (AD7.2), a literature research and a concept for a sample quality study on impact of thawing and freezing on integrity of human samples was conducted. For budget estimates and to support the overall planning of the analytical work, tentative prices for biomarker analysis in HBM4EU were obtained within WP9.2, Network of Reference HBM laboratories for performing biomarker analysis, developing new methods, and supporting the QA/QC program at EU level For the Quality Assurance/Quality Control Scheme in the HBM4EU project (ICI/EQUAS) (WP9.4), the rounds 1-3 of proficiency testing for the determination of PFAS in serum including PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFBS, PFHxS, PFHpS, PFOS (sum of all isomers) were concluded. The number of qualified laboratories for PFAS analysis after the 3rd round comprises 21. Based on existing HBM data, D10.4 presents the first annual list of European reference values (ERV). From the existing European data collections studies including analyses of PFOS, PFOA, PFNA and PFHxS dated between 2008 and 2015, were selected. Aggregated data for the selected PFAS were reported for 4 birth cohorts, with measurements in cord blood plasma or cord serum. One study reported blood plasma levels of children, one study blood serum levels of teenagers, and 6 studies measured blood serum/plasma levels of adults. All studies included at least 100 participants. Despite variations in design, populations, analytical methods, and geographic location, the median concentrations in the different European studies are rather similar, with ratios between the highest and lowest median concentration always being less than 10. In the table below the reported median-values and 95th percentiles of the individual studies were averaged (by taking the median) over the different studies of newborns, children & teenagers combined, and adults. These levels support the concentration levels reported in the recent EFSA opinion on the risks to human health related to the presence of perfluoralkyl substances in food, (EFSA 2020). The individual data collections prepared and made available within HBM4EU also contained aggregated data stratified by age, sex and educational level. From these stratifications it can be seen that the PFAS concentrations are in general higher in men compared to women for the teenager and adult studies. This could probably be explained by the elimination of PFASs through menstruation, and for mothers also through delivery and lactation. Also, there seems a trend that participants with higher educational level have higher exposure levels compared with low to medium educational level. This trend was however less obvious and could not be observed in all studies and for all compounds. In some studies, higher levels of PFASs were observed with increasing age, indicating possible cumulative exposure over time. This was however not observed for all studies and for all compounds. For PFAS, an overview of how to address the exposure related policy questions was generated and reported in D10.5. Further, an update of the Statistical Assessment Plan (SAP) is under development in D10.10, including plans for the assessment of PFAS measured in the aligned studies in teenagers, which comprises European exposure levels, exposure distributions, geographical comparisons,

Policy Question	Short Summary of Results
	exposure determinants, exposure-effect associations (BMI and metabolism, sexual maturation, asthma and allergy), and exposure-effect biomarker – health effect path analysis (sexual maturation and metabolism). For the investigation of PFAS levels and exposure determinants in vulnerable population groups (newborns and pregnant women) in WP10.4, Data analysis including the generation of European reference values 11 existing European birth cohorts were identified (from Norway, Denmark, Belgium, France, Spain and Slovenia). The related individual data are shared bilaterally, and the next steps will be the initiation of the statistical analysis. D10.6: overview on aggregated data of existing HBM data collections obtained through HBM4EU. These data and the plots generated will be used for further interpretation. In addition, these data will be integrated into IPCheM. Within WP13.3, Implementing mechanistic toxicology for risk assessment studies for the examination of exposure-health relationships were identified. For this, a dynamic inventory of existing studies was made. PFAS studies included in the inventory comprise 10 studies in pregnant women (all studies contain information on health outcomes), 18 studies in children and adolescents (out of which: 12 prospective cohort studies), and 13 studies in adults and the elderly (out of which: 5 prospective cohort studies). In the annual work plan 2018 is was outlined to merge individual data from several birth cohorts and to examine the associations between PFAS and low birth weight, and to examine associations with birth weight as continuous outcomes, especially for PFAS other than PFOS and PFOA. Thus, a detailed research protocol was written, and partners who agreed to share their individual data were identified. Additionally, it was decided to conduct similar analyses by merging individual data for PFAS to assess the associations between maternal PFAS concentrations and thyroid function in both mothers and their newborn. Several partners agreed to share their data. Reporting the preliminary results is anticipated in M36. Within WP5.3 (D5.5), the use of HBM data in risk assessment (RA) and health impact assessment (HIA) strategies was demonstrated (further description of this work see policy question 11, concerning PFAS mixtures). The EFSA opinion on PFOA and PFOS (EFSA 2018) was used as starting point. According to EFSA the exposure of a considerable part of the European population exceeds the provisional tolerable weekly intakes also in case of using low exposure estimations. These exceedances could be also observed by comparison of internal human benchmark dose levels with levels detected in Human Biomonitoring studies from Europe. The Benchmark dose levels were based on increase of cholesterol, delayed response to vaccination in children and reduction in birth weight (EFSA, 2018). D5.5: The PFAS risk assessment was based on the EFSA opinion 2018 on PFOS and PFOA, in which HBM data was used for exposure and risk assessment. In addition, other recent risk assessment for PFAS were considered. In WP 5.3 the focus was laid on the mixture risk assessment of PFAS. Thus, a preliminary mixture risk assessment of four PFAS was conducted including PFOS, PFOA, PFNA and PFHxS since these substances are major PFAS in human tissues. Results of the mixture risk assessment showed great uncertainties, a major one were due to species differences with respect to the toxicokinetics and toxicodynamic, which are specifically relevant for PFAs because of their unique properties. When the mixture risk assessment is based on animal data solely, the hazard index is usually <1, which indicates no potential risk. However, when the mixture risk assessment is conducted based on HBM data from different European regions, the human-based hazard data for PFOS and PFOA comprising cholesterol increase, and the extrapolated hazard data using animal-based relative potency factors for PFNA and PFHxS, the hazard index is >1. Overall, there were three main conclusions. (1) There is a need for human-relevant hazard and HBM data, and there are also data gaps for the majority of the 4,000 PFAS currently used related to uses, exposure patterns and toxicity. Especially, human-relevant exposure and hazard data for PFAS apart PFOS and PFOA is needed. (2) There is a need for endpoint-specific relative potency factors based on internal doses in human. (3) There is a need for more intensive collaboration between toxicologists and epidemiologists to raise risk assessment to a higher level. Further, there are some other

Policy Question	Short Summary of Results
	important issues, including e.g. how to handle vP substances in risk assessments, the need for new methods and approaches for the grouping of substances, and prediction of their toxicity and the validation of this methods. According to the preliminary mixture risk assessment performed in WP 5.3 the current exposures seem to exceed the Guidance values for PFAS in some parts of the EU population. This is supported by the recent EFSA opinion (EFSA, 2020), which concludes that based on the estimated lower bound exposure, but also reported serum levels, the CONTAM Panel that parts of the European population exceed the derived tolerable daily intake, which is of concern. AD5.3: in the HBM4EU repository and IPChEM, some harmonised datasets (however, a limited number) are currently available. The avaibale datasets include aggregated data on exposure biomarker levels for FOSA, N-EtFOSA, N-MeFOSA, PFBA, PFBS, PFDA, PFDoDA, PFDS, PFHpA, PFHxA, PFHxS, PFNA, PFOA, PFOS, PFPeA, PFTeDA, PFTrDA and/or PFUnDA, for the matrices serum, cord serum and/or breast milk. The datasets belong to studies from Belgium, Czech Republic, Slovakia and Demark and were collected within 2000-2017. Available stratifications include information on sex, age groups (including infants <1 yr), ISCED, current non-smokers, and current non-smokers vs smokers. Impact indicators will be established and used to answer the policy questions. Considerations for future: There is an need for extending analytical methods to include more substances (PFAS) in the existing methods, such as short-chain PFAS (PFBA, PFOeA, trifluoroacetic acid (TFA)) as well as intermediates from of fluorotelomer precursor PFAS (FTCAs, FTUnCAs) which may have a different toxicity compared to PFAAs. In addition, the implementation of analytical methods for the analysis of “total” PFAS is needed (this would be also future policy relevant for PFAS restriction and the EU PFAS strategy).
2. Are there differences in exposure of the EU population to regulated and non-regulated PFASs?	As described above (policy question 1) efforts are underway to characterise the exposure of the EU population to PFAS, including the alignment of studies, the development of a post-harmonisation strategy, as well as the examination of exposure-health relationships and the inclusion of HBM data in risk assessment. Differences in exposure levels of the measured regulated and non-regulated PFAS will be assessed as well. AD5.3: HBM indicators that display HBM levels for regulated and not yet regulated PFAS can be displayed if enough data become available in the HBM4EU repository/IPCHEM.
3. Has restriction of PFOS according to the POP Regulation led to a reduction in exposure, especially for children?	HBM4EU aligned studies on PFAS will focus on teenagers. Thus, no new data on exposure of children will become available. Within HBM4EU time trends will also be further explored within WP 10 using existing data, though limitations due to different study populations and geographical areas need to be considered. Within WP13.2 observations from birth cohorts will also bring further evidence. AD5.3: An indicator can be displayed based on aggregated HBM data stratified for children, and time trends may illustrate success or failure in reduction. Concerning time trends observed in Human Biomonitoring studies the EFSA opinion on PFOS and PFOA in food states that generally, after the year 2000, the concentrations in serum/plasma of PFOS, PFOA and in some studies PFHxS have decreased, while the concentrations of PFNA, PFDA and PFUnDA have increased. No clear trends have been reported for the remaining PFAS (EFSA, 2020).
4. Is exposure driven by diet, consumer exposure,	Within WP12 food intake was found to be the most important contributive route to the exposure of PFOS and PFOA, with percentages of 97% and 98% of the total intake, respectively. These estimations were made based on a study from Catalonia (D12.1). Dietary exposure to PFOS and

Policy Question	Short Summary of Results
occupation or environmental contamination?	PFOA, PFNA and PFHxS has been thoroughly assessed by EFSA. (EFSA 2018, 2020). Despite exposure in hot spot regions or specific occupational settings, diet is the main source of PFAS exposure. In WP8.5 concerning targeted occupational studies the aim is to bridge gaps of knowledge in occupational exposure. Thus, EU relevant data on occupation-related exposures to prioritised substances are collected in critical occupations by using harmonised methods and questionnaires. A targeted occupational study on hexavalent chromium (Cr(VI)) is conducted. In addition to chromium analysis, samples are also collected for PFAS analysis, as these substances are used in chromium plating. Sampling is under way. The analysis of PFAS will be done if the ICI/EQUAS round for PFAS will be completed (WP9.4). The present study also includes the analyses of several effect biomarkers which are made mainly with the participants own funding. It is expected that the study will be completed by the end of 2019. In D7.7, template materials to support the participation in HBM4EU surveys were developed. The template materials were transformed into tailored materials for each HBM4EU survey, which were translated into different languages. Specifically for PFAS, materials were developed related to the first aligned occupational HBM4EU survey (Exposure of European Workers to Hexavalent Chromium (Cr(VI)) and other chemicals), whereas targeted materials are available in English, German, Finnish, French, Italian, Dutch, Polish and Portuguese comprising the participating countries. Additionally, as stated in the answer to policy question 1, different work has been undertaken for the identification of the exposure of the EU population to PFAS. As described above exposure of European teenagers will be investigated, this will be accompanied by the assessment of determinants of exposure. AD5.3: IPCHEM contains substance specific data in food items, consumption data and environmental data. These can be displayed next to the specific HBM data, preferentially at the same aggregation level. The data will have to be checked for harmonisation and compliance with the quality criteria, before a potential indicator on the route of exposure is done. In the Additional Deliverable AD12.3: Exposure model testing results, a generic PBPK model was used to assess the concentration of PFASs in human tissues, based on an existing model previously validated for PFOS and PFOA. Experimental data on PFAS concentrations in human tissues from individuals in Tarragona County (NE of Spain) were used to estimate the values of some distribution and elimination parameters needed for the simulation.

Policy Question	Short Summary of Results
	 **Figure 34: (a) Dietary intake of total PFASs by the population of Catalonia. (b) Intake of PFOA and PFOS by the population of Catalonia.** D12.7: Report on optimised sampling schemes for rapidly metabolised and persistent/biocumulative substances. According to literature, PFAS main exposure sources are dietary products (specifically fish products), contaminated water, soil and dust. For newborns, the main source of exposure is breast milk. Exposure scenarios for PFAS were established in WP 12.1 (Integrated exposure modelling aiming at proving input for policy questions for the 1st and the 2nd set of priority substances). Scenario 1 includes lifetime exposure through diet and drinking water for PFOS and PFOA. Both substances reach steady-state levels in blood and urine after 20-30 years. The blood levels are three orders of magnitudes higher in blood compared to urine. The intra-day variability is difficult to be captured. Due to the long half-times in humans, the intra-day variability of exposure cannot be reflected in the body fluids. For the investigation of time-trends, one blood sample per year is sufficient.
5. Which areas and environmental media in Europe are contaminated with PFASs?	Within WP10 a substance-group-specific statistical analysis plan has been developed. Variables for assessing environmental contamination have been identified: place of birth, place of residence (near a fluorochemical industrial facility, near civilian airports, military bases, wastewater treatment facilities, or firefighting training facilities, near agricultural areas characterised by the use of soil conditioners),years of residence, consumption of tap water, use/consumption of groundwater or surface water, locally produced food, own grown vegetables, own raised livestock, fish and seafood from a local body of water. Primary focus of HBM4EU is exploring the background exposure of the general population and no specific studies in known hotspot areas are planned. Though HBM4EU study materials can be used in national studies performed to investigate certain contamination cases. AD5.3: This cannot directly be answered but result indicators for regional HBM data can inform about geographical exposures. Further, several partners in HBM4EU are involved in studies investigating exposure in contaminated regions, the results of these studies will be used to answer the respective HBM4EU policy questions.
6. How can this feed into an assessment of the TDI for	Suitable PFAS studies for the examination of exposure-health relationsships were identified within WP13.3, including studies in pregnant women, children and adolescents, adults and the elderly. Additionally, it is planned to merge individual data from several birth cohorts for the examination

Policy Question	Short Summary of Results
PFOS and PFOA set by EFSA?	of associations of PFAS exposure and low birth weight, and birth weight as continuous outcome. A similar investigation will be conducted for the assessment of associations between PFAS and thyroid function in mothers and their newborn. Results are expected to be reported in M36. For more detailed information see answer to policy question No. 1. AD5.3: Result indicators can be constructed to display geographical differences and time trends using HBM levels. This will be done when harmonised and comparable data are available within HBM4EU repository/IPCHEM. An additional deliverable has been prepared within WP5, Translation of results into policy: 'Timelines of Opportunity', submitted to the coordinator) in which a strategy is being proposed to systematically map both the 'policy timeline(s)' and 'HBM4EU timeline(s)', in order to identify potential windows of opportunity for policy uptake. (Lead by UAntwerp, EEA and VITO, in collaboration with other partners including EAA (CGL PFAS)). The strategy takes into account different types of HBM4EU output as well as different types of policy processes that might benefit from HBM data. Furthermore, HBM4EU has contributed to the public consultation of the recent EFSA opinion on PFAS, 2020 by submitting feedback and comments. Experts of the CONTAM panel presented and discussed the essential parts of the opinion with the HBM4EU PFAS community per webex on April, 7. Results of the aligned studies of HBM4EU will feed into the policy processes identified, including the broad restriction of PFAS under REACH but also the chemical strategy for sustainability (toxic free environment).
7. What is the impact of a pending 2016 ECHA decision to restrict the manufacturing, marketing and use of PFOA under REACH?	New data which will become available within HBM4EU and can be compared with European data from earlier studies. As stated above concerning time trends observed in Human Biomonitoring studies the EFSA opinion on PFOS and PFOA in food states that generally, after the year 2000, the concentrations in serum/plasma of PFOS, PFOA and in some studies PFHxS have decreased, while the concentrations of PFNA, PFDA and PFUnDA have increased. No clear trends have been reported for the remaining PFAS (EFSA, 2020). This demonstrates, that the restriction of certain PFAS leads on one hand to decreases of these PFAS, but to increases of potential substitute PFAS. It is of utmost importance to avoid regrettable substitutions.
8. Is it important to eliminate legacy PFASs from material cycles (i.e. waste electronic equipment) when implementing a circular economy in order to protect human health?	This policy questions goes beyond the research focus of HBM4EU. However, due to the long half-life in humans, the exceedances of tolerable daily intakes and internal benchmark dose levels of substances which are already restricted such as PFOS and PFOA it seems indicated to eliminate PFAS from material cycles when implementing a circular economy in order to protect human health.

Policy Question	Short Summary of Results
9. Can differences in PFASs profiles be observed in different population groups and time periods?	Efforts to assess PFAS exposure within HBM4EU are described above. Differences in PFAS profiles could be described by analysing time trend studies, which are not available at European level so far. European time trend studies will not be possible within HBM4EU, though they could be initiated. At the MB meeting of November, a decision memo concerning the proposal to extend the analytical spectrum of PFAS compounds (including the total extractable fluorine content in order to identify the amount of currently unidentified PFAS) in a subset of samples by selected labs was presented. The MB acknowledged the importance of the proposal but pointed out that with regard to budget, workload of WP9 and timeline it is not possible to integrate it into HBM4EU. But it would be desirable to get a review about the state of the art and a recommendation for a future project.
10. What are the PFASs levels and health effects in vulnerable population groups?	As described above, PFAS exposure will be examined in European teenagers. Within WP 14.2, Selection of biomarkers of effect according to their utility in human studies (D14.3) biomarkers of effect according to their utility in human studies were selected. Though it is unclear which of them will and can be actually measured in the HBM4EU aligned study it was proposed to measure brain derived neutrophic factor, thyroid hormones and glucose markers, serum lipids and adipokines, beside neurobehavioural tests and antropometric tests. In WP 13.1. Knowledge base on causal pathways from chemical exposure to health outcomes (Adverse outcome pathways) work on characterisation of the key events in the AOPs for disruption of cholesterol/lipid metabolism and inflammatory responses with links to cardiovascular disease; collection of information on potential mechanisms beyond PFAS-induced effects on birth weight and immune toxicity is ongoing. In D 13.4 report on AOPs of priority substances first results addressing these endpoints were compiled. In D13.5. gaps for the establishment of AOPs were identified, and the need for required studies described. Suitable studies for the examination of exposure-health relationships of PFAS were identified within WP13.2. Health effects in humans based on birth and adult cohorts (D13.3) including studies in pregnant women, children and adolescents, adults and the elderly. Additionally, it is planned to merge individual data from several birth cohorts for the examination of associations of PFAS exposure and low birth weight, and birth weight as continuous outcome. A similar investigation will be conducted for the assessment of associations between PFAS and thyroid function in mothers and their newborn. Results are expected to be reported in M36. The additional deliverable AD13.3, which will be published soon, provides a concise summary on current progress, achievements as well as future plans in Task 13.2 cohorts within HBM4EU time frame, and also beyond. The recent draft scientific opinion (EFSA 2020) points out that toddlers and other children had approximately two-fold higher mean intake than older age groups (adolescents, adults, elderly, very elderly). The CONTAM Panel concluded further, that parts of the European population exceed the tolerable weekly intake, TWI, which is of concern. Therefore, it can be assumed that toddlers and children are a vulnerable population group and are exposed to PFAS levels, which are cause of concern.
11. How can mixture effects of environmental and human PFASs mixtures present to	As described above (Policy Question 10) Suitable PFAS studies for the examination of exposure-health relationships were identified within WP13.2 (D13.3.) including studies in pregnant women, children and adolescents, adults and the elderly. Additionally, it is planned to merge individual data from several birth cohorts for the examination of associations of PFAS exposure and low birth weight, and birth weight as continuous outcome. A similar investigation will be conducted for the assessment of associations between PFAS and thyroid function in mothers

Policy Question	Short Summary of Results
date be estimated?	and their newborn. Results are expected to be reported in M36. For more detailed information see also Policy Question No. 1. Within WP 5.3. Inclusion of HBM guidance and reference values (HBM GVs and HBM RVs) in risk assessment/health impact assessment strategies the use of HBM data in risk assessment (RA) and health impact assessment (HIA) strategies was demonstrated. First attempts to assess mixture effects of PFAS have been undertaken in (D5.5). The challenges to date for assessing mixture effects of PFAS are the lack of endpoint specific toxicity data for PFAS preferable in humans as well as human exposure data. The EFSA opinion on PFOA and PFOS dated 2018 was used as starting point. According to EFSA the exposure of a considerable part of the European population exceeds the provisional tolerable weekly intakes also in case of using low exposure estimations. Within the work under WP5.3 (D5.5.) PFOA, PFOS, PFNA and PFHxS were considered for the risk assessment in the general population, whereas cholesterol increase in humans for PFOS and PFOA, and hazard data based on animal data for PFNA and PFHxS (developmental effects and thyroid follicularcell damage) used to derive minimal risk levels by ATSDR, were used for the RA. When conducting the mixture RA for PFAS, great uncertainties were identified stemming from species differences with regard to toxicokinetics and toxicodynamics. This is specifically relevant for PFAS because of their unique properties. The mixture RA conducted based on animal data only indicated that there is no potential risk. However, the mixture RA conducted based on European HBM data using epidemiological data (cholesterol increase) for PFOS and PFOA and extrapolated hazard data using animal-based relative potency factors for PFNA and PFHxS indicated a potential risk of parts of the population to these substances. The mixture risk assessment of EFSA, on PFOS, PFOA, PFNA, PFHxS indicated also that there is a potential risk of parts of the population to these substances. Within WP13.1 work on adverse outcome pathways is ongoing. Selected effects for contribution to the OECD AOP framework were based on the endpoints for which benchmark dose levels were derived by EFSA: effects on the liver accompanied by increase in cholesterol levels, effects on birth weight and effects on the immune system, all of those based on human data (D13.4). Certain AOPs in the AOP wiki database were identified which could be relevant for PFAS exposure in humans, however considerable data gaps related to causality and mode of action are lacking. Though, the mechanistic pathway from PFAS exposure to adverse health outcomes will be further explored. Within WP 14 effect biomarkers for PFAS were successfully established. Placental extracts (alpha fractions), containing mixtures of persistent and lipophilic chemicals, showed significant anti-androgenic activity. The hormonal profile from placental tissue was quantified, as well as some epigenetic markers such as Histone H2AX phosphorylation (Gamma-H2AX), trimethylation of histone 3 at lysine (H3K4me) and DNA methylation of BDNF, in addition to untargeted metabolomic analysis. Finally, 8OHdG levels were assessed in urine samples coupled to the placentas from the same women. This work has shown that chemical mixtures isolated from human samples can be assessed, and its biological activity quantified using different biomarkers cell based tools. Placenta tissue could be used as a relevant biological matrix to assess both exposure and effect biomarkers. The placenta can also be used to explore the implementation of novel effect biomarkers in Human Biomonitoring programs, due to the volume and availability of this biological sample. Future work will focus on the relationship between exposure and effect biomarkers tested in placenta samples (included in D14.4 and AD14.4); ii) further to assess the implementation of the most appropriate biomarkers of effect and combined effects in other biological matrices more frequently recruited in HBM programs, such as blood and urine; to explore the concentration to specific chemical families, such as PFAS and metabolites, in placenta and serum samples, in order to assess its combined effects using different effect biomarkers such as some in vitro cell bioassays. In WP14, an inventory of available effect biomarkers was created. Positive associations with ASD/ADHD and behaviour in children were identified in several studies, however, data is inconsistent. Data on negative effects on developmental milestones are also inconsistent. In several case-

Policy Question	Short Summary of Results
	studies associations with e.g. breast cancer, prostate cancer and colorectal cancer were found, but more prospective studies are needed. Related to endocrine disorders, lower testosterone and oestradiol levels as well as thyroid hormone disruptive potential were identified. There are also indications of immunosuppressive effects in several studies, as well as positive associations with asthma and allergy. Further, prenatal PFAS exposure is associated with higher fat percentage in children leading to an increased risk of overweight/obesity, and there are associations to glucose homeostasis, dyslipidemia, high cholesterol, metabolic syndrome and risk of diabetes. Related to reproductive disorders, positive associations with preeclampsia and pregnancy hypertension were identified. Further, prenatal PFAS exposure may results in a delay of menarche and may cause abnormal menstruation/length. Prenatal exposure can also reduce birth weight, length, APGAR score and change gestational length. Decreases in semen quality and sperm count were also found. Additionally, in one study correlations with anogenital distance in girls and a risk of cerebral palsy in boys have been shown. The development of new analytical methods for the assessment of FPAS mixtures in serum and placenta samples are ongoing. In addition, there are currently activities related to the assessment of the biological effect of PFAS mixtures using in vitro biomarkers of combined activity, related to the generation of new knowledge on AOPs by in vitro research for the mechanism for PFAS on liver-cholesterol and lipid metabolism and intracellular levels of PFAS, and related to the identification of exposure-health associations from a cohort study (human milk biobanking). AD14.5: A methodology was developed to extract and fractionate the real mixture of PFAS from human serum samples. In 702 Danish females it was demonstrated that PFAS induced xenoestrogenic transactivity is significantly inverse related to birth weight, length and head circumference. Further, combined mixture of PFAS from placenta homogenate sample provided by the Spanish INMA-Granada cohort was isolated and xenoestrogenic transactivity was measured. Similar as for the serum samples, 52% of the placenta extracts significantly induced xenoestrogenic transactivity, and 68% further enhanced the transactivity of the natural E2 receptor ligand. In addition, a literature review on PFAS exposure and thyroid homeostatis in epidemiological studies was conducted. Maternal thyroid hormones are essential for fetal brain development and PFAA are suggested to interfere with these hormones in 2nd or 3rd trimesters. In addition, PFAS exposure may cause hypothyroid homeostasis.

Policy Question	Short Summary of Results
12. How can PFAS substances of relevance for human exposure and health be identified having in mind that more than 3000 substances are at the market?	For the majority of the 4,000 currently used PFAS considerable data gaps exist related to current uses, exposure patterns and toxicity. Besides regulatory action which is called for by member states and the European Commission also research is needed. HBM4EU PFAS experts have submitted a statement to the public consultation on the draft scientific opinion on the risks to human health related to the presence of perfluoroalkyl substances in food, which states among others: There is a need for Human Biomonitoring data for PFAS other than those addressed in the risk assessment (specifically those which are used/formed in high volumes as a result of substituting legacy PFAS). There is a need to measure the total organic fluorine content in humans in order to assess the magnitude of the so far unknown or not yet assessable contribution of PFAS in humans. More longitudinal epidemiological PFAS studies are needed. Research on immunotoxicity, endocrine disruption and birth outcomes is required. Research on other toxicological endpoints is also needed including effects on the lungs/respiratory system from prenatal exposure, and cancers such as breast cancer in adults. Research on adverse outcome pathways is needed. To support the science-based grouping of PFAS, a better understanding of the modes of action of different PFASs is needed. Further studying relative potencies of PFAS for mixture risk assessment would be of added value.
13. How can identification and assessment (including data on (potential) adverse effects on human health and the environment) of alternatives currently hampered by CBI (Confidential Business Information) be facilitated?	--
14. How much are HBM values dependent on host characteristics and does this have implications for identifying vulnerable groups?	A new PBPK model for PFOA and PFOS was developed based on a previously reported model within WP 12. (D12.1). For validation purposes, data on PFOA and PFOS in human tissues from people living in the area of study (Tarragona County) were used. The levels of 13 PFASs, including PFOA and PFOS, were reported in blood samples of 48 residents in that same area. In addition to the model validation, a study on the best partition coefficients was conducted. Hence, the model was tested by using, as input data, partition coefficients from studies conducted with either rats or humans. Data sets were compared to detect any improvement in the performance of both original and adapted PBPK models. Several discussion points have been identified, e.g. highlighting the importance to obtain partitioning data from humans and of PFAS levels in human tissues in order to refine the model. It could be further assessed if the model could be used to explore this question further. In AD12.3: integrated exposure models for PFAS exposure scenarios are described. A PBPK model for PFAS was used for the prediction of internal tissue dose. For testing the model, data on general adult population from Spain, and a cohort of pregnant women and ongoing birth

Policy Question	Short Summary of Results
	cohort from Spain were used. In addition, a study was used investigating PFAS in most widely consumed foodstuffs in Spain as well as the total dietary intake of PFAS. In this AD12.3, exposure estimates were derived based on the consumption study and they were used as input for the PBPK model to estimate the concentration of 11 PFAS in human tissues. PKs were estimated by using data on PFAS concentrations in plasma and autopsy tissue samples. PK values ranged from 0.001 for PFDS, PFDA and PFTeA in liver as well as for PFTeA in bone marrow to 201.6 for PFHxA in brain. For model parameterisation, data on PFAS concentrations in blood and human tissues from three studies were used. The simulations followed a trend: nearly linear at the beginning, reaching a plateau after 20-30 years. The simulation results found in tissues depended on the PK as well as on elimination constants and daily intake. The highest concentrations in liver corresponded to PFOS, PFHxA, PFHpA, PFOA and PFNA ranging between 1.32 ng/g (PFNA) and 127.6 ng/g (PFOS). In contrast, the minimum values of other long-chain PFAS (PFDS, PFDA, PFTeDA, PFFUnDA) were also found in liver. These findings were surprising, because the liver is usually considered to be the main target organ were PFAS accumulate. In addition, the highest PFHxA concentration was found in brain. The kidney showed a similar PFAS profile to those in plasma and liver whereat PFOS was the main contributor (113.4 ng/g). For the assessment of the concentration of PFAS in human tissues, a generic PBPK model was used based on an existing model previously validated for PFOS and PFOA. Bacause of the scarcity of PK and PD data for PFAS other than PFOS and PFOA, differences among the other substances were not be studied in depth by the PBPK models. Thus, further well-conducted HBM investigations are necessary. In general, the assessment of the models showed that there is a lack in data supporting integrated exposure, and another significant problem is the data quality. Further, there is a need for the detailed description of exposure mechanisms that are not straightforward such as inhalation or food ingestion. AD12.5: PBPK model was used for exposure reconstruction. Data used was from the Tarragona cohort study, and only PFOS and PFOA were considered. Exposure estimates: Daily intakes were estimated with a constant oral exposure scenario. The mean daily intakes estimated for adult population (assuming 70 kg bodyweight) were 24.43 ng/kg bw/d for PFOS and 5 ng/kg bw/d for PFOA. At steady-state, an exposure conversion factor for plasma levels of 0.008 for PFOS and of 0.0082 for PFOA were estimated. Dietary intake estimates from HBM data exposure reconstruction: median intakes for PFOS estimated ranged between 0.076 µg/kg bw/d (Germany) and 0.172 µg/kg bw/d (Denmark, and median intakes for PFOA ranged between 0.019 µg/kg bw/d (Norway) and 0,037 µg/kg bw/d (Denmark). The exposure reconstruction offers unique opportunities related to HBM data interpretation. For these reconstructions a minimum information related to toxicokinetic behaviour of a substance (and its metabolites) is required, which allows a translation of measured biomarker levels at a given time-point with long-term daily intake patterns. Ongoing work in WP12 concerns a dynamic age and gender specific PBPK model, and paediatrics case study.