Prioritized substance group:

Per-/polyfluoroalkyl substances (PFASs)

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Short overview of results of the activities carried out within HBM4EU in 2019 to answer the policy questions with reference to corresponding deliverables

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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 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 between 2014 and 2019 related to three age groups. The strategy includes countries from different European regions focusing 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. Data will be collected in countries of all parts of Europe: Norway and Sweden (North), Slovakia (East), Slovenia, Spain, Greece (South) France, Belgium, Germany (West). Up to 12 PFAS will be measured in the respective samples: PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFBS, PFHxS, PFHpS, 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. Within task 7.6, a post-harmonisation strategy for the collection of data (questionnaires) was required. For the collection of new samples and data WP7.3 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 developed including additional specific single questionnaires for PFAS. In WP 8.4, influencing and interfering factors for sampling and storage were identified, whereas for each substance group recommendations were made to

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	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. PFDS, PFHxS, perfluorooctanoate and perfluorononanoate are stable in serum for at least 10 days stored at room temperature, and for at least 8 months stored at -20°C or below. Most PFSAs and PFCAs measured in water solution are stable for three months when stored at 4°C, but are adsorbed to the surface of PP containers rapidly, thus indicating the use of other sample containers like e.g. high-density PE containers even for short-term storage. 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. For PFAS, the range (min-max) of time spans required for the analysis of 300 samples were evaluated to be between 1 and 8 months depending on the specific laboratory responding to the request that have been made in this AD. The range of prices provided (by n=14 labs) for selected PFAS (and partially for additional PFAS) ranged between 70 and 641 € per sample (19,500 – 67,500 € for 300 samples). Overall, 21 labs were contacted to provide information, 14 of which responded. For the Quality Assurance/Quality Control Scheme in the HBM4EU project (ICI/EQUAS) (WP9.4), the first and second round 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 third round has started. Based on existing HBM data, D10.4 presents the first annual list of European reference values (ERV). First steps were made to calculate exposure distributions for existing HBM data on PFAS. Research protocols were developed. Currently, no harmonised aggregated data are available yet. As first results for PFAS, available HBM data from existing data collections from different time periods (without any limitations) and across different geographical European regions were collected. The majority of HBM data on PFAS is available from birth cohorts leading to a primary focus on this population group. 17 data collections were identified for PFAS analysis in maternal plasma/serum during pregnancy or in cord plasma/serum of the newborn. 14 of the respective data owners were invited to share their data for joint analyses. Data collections with too small sample (<120) were excluded. Until date of publication of D10.4 (12/2018), the owners of 10 data collections were willing to participate and 5 were interested in participating but needed approval from their authorities. Results are planned to be published in 2019. Within WP13.3 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

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	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. These data were collected. The results for PFAS are expected to be reported in M36. Additionally, it was decided to conduct similar analyse by merging individual data for PFAS to assess the associations between maternal PFAS concentrations and thyroid function in both mothers and their newborns. 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 answer concerning 11 PFAS mixtures). The recent EFSA opinion on PFOA and PFOS was used as starting point. According to EFSA the exposure of a considerable part of the European population exceedsexceeds 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 detecteddetected 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).
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 characterize the exposure of the EU population to PFAS, including the alignment of studies, the development of a post-harmonisation strategy, the planning of the analytical work, 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.
3. Has restriction of PFOS according to the POP Regulation led to a reduction in exposure, especially for children?	Within HBM4EU aligned studies on PFAS the selected age group are teenagers. Thus, no new data on exposure of children will become available. Within HBM4EU time trends could 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 could also bring further evidence. Concerning time trends observed in Human Biomonitoring studies the EFSA opinion on PFOS and PFOA in food states: Following an increase from the early 1970s, decreasing concentrations of PFOS and PFOA have been observed in many time trend studies after the year 2000 (EFSA, 2018).
4. Is exposure driven by diet, consumer exposure, occupation or environmental contamination?	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 PFOA has been recently thoroughly assessed by EFSA. (EFSA 2018).

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	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 WP7.7, 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, including the alignment of studies, the development of a post-harmonisation strategy, the planning of the analytical work, as well as the examination of exposure-health relationships and the inclusion of HBM data in risk assessment. As described above exposure of European teenagers will be investigated, this will be accompanied by the assessment of determinants of exposure.
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. However, 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.
6. How can this feed into an assessment of the TDI for PFOS and PFOA set by EFSA?	Suitable PFAS studies for the examination of exposure-health relationshipsrelationships 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 of associations of PFAS exposure and low birth weight, and birth weight as continuous outcome. A similar investigation will be conducted for the assessment

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	of associations between PFAS and thyroid function in mothers and their newborns. Results are expected to be reported in M36. For more detailed information see answer to policy question No. 1.
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. According to EFSA, 2018 also a decrease in PFOA could be observed in Human Biomonitoring studies, including time trend analyses (EFSA, 2018)
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?	Due to the long half-life in humans, to 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.
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.
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 (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 actuallyactually 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. 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, and 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 newborns. Results are expected to be reported in M36. For more detailed information see policy question 1. Further, it can be referredreferred to the EFSA assessmentassessment, compiling Human Biomonitoring and benchmark dose levels. The table below depicts serum concentrations of PFOS and PFOA in European adult and children populations based on medians derived from studies reported in

<table>
<tr><th>Policy Question</th><th>Short Summary of Results</th></tr>
<tr><td></td><td>EFSA 2018 and the comparison with the derived BMDL5 levels by EFSA as reported in WP5.3 (D5.5.)
<table>
<tr><td>Age group</td><td colspan="2">Adults</td><td colspan="2">Children</td></tr>
<tr><td>Concentration</td><td colspan="4">ng/ml</td></tr>
<tr><td></td><td>PFOA</td><td>PFOS</td><td>PFOA</td><td>PFOS</td></tr>
<tr><td>BMDL5</td><td>9.2-9.4</td><td>21-25</td><td>9.2-9.4</td><td>10.5</td></tr>
<tr><td>Median*</td><td>1.9</td><td>7.7</td><td>3.3</td><td>3.2</td></tr>
<tr><td>Mean*</td><td>2.1</td><td>7.5</td><td>3.3</td><td>3.3</td></tr>
<tr><td>Minimum*</td><td>0.76</td><td>1.7</td><td>0.49</td><td>0.49</td></tr>
<tr><td>Maximum*</td><td>4.9</td><td>27.4</td><td>6.9</td><td>8.6</td></tr>
<tr><td>Number of studies</td><td>32</td><td>32</td><td>8</td><td>8</td></tr>
</table>
The critical effect (benchmark dose level benchmark dose for a 5% increase: BMDL5) identified in EFSA 2018 was the increase of serum cholesterol (9.2-9.4 ng/ml). Other benchmark dose levels established were delayed response to vaccination for children for PFOS: BMDL5: 10.5 ng/ml and effects on birth weight: PFOA: BMDL5: 4-10.6 ng/ml, PFOS: 21 ng/ml). Using individual data, for PFOS, the concentrations in adults and children ranged from 0.06 to 392 ng/mL and from 0.47 to 23 ng/mL, respectively.
For PFOA, the concentrations in adults and children ranged from 0.03 to 81 ng/mL and from 0.45 to 19.5 (P95) ng/mL, respectively. Much higher concentrations of PFOS and PFOA are also the case for occupationally exposed adults & for persons experiencing elevated exposure from for instance contaminated drinking water.</td></tr>
<tr><td>11. How can mixture effects of environmental and human PFASs mixtures present to date be estimated?</td><td>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 and their newborns. Results are expected to be reported in M36. For more detailed information see also Policy Question No. 1.

First attempts to assess mixture effects of PFAS have been undertaken in WP 5.3. (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. Currently EFSA is assessing the risk of PFAS mixtures, it is</td></tr>
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	however not clear which PFAS will be included and which endpoint in which species will be selected. Within WP 5.3 (D5.5.) the use of HBM data in risk assessment (RA) and health impact assessment (HIA) strategies was demonstrated. The recent EFSA opinion on PFOA and PFOS was used as starting point. According to EFSA the exposure of a considerable part of the European population exceedsexceeds the provisional tolerable weekly intakes also in case of using low exposure estimations. Within work under WP5.3 (D5.5.)PFOA, PFOS, PFNA and PFHxS were considered for the RA in the general population, whereas cholesterol increase in humans for PFOS and PFOA, and hazard data based on animal data for PFNA and PFHxS, 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. Based on this work, the following conclusions were drawn: (i) there is a need for human-relevant hazard and HBM data, in order to establish endpoint specific hazard indices with human relevance. For the majority of the 4,000 currently used PFAS considerable data gaps exist related to current uses, exposure patterns and toxicity. Especially, human-relevant exposure and hazard data is needed for PFAS apart from PFOS and PFOA, (ii) there is a need for endpoint specific relative potency factors based on internal doses in humans, and (iii) there is a call for more intensive collaboration between toxicologists and epidemiologists to raise RAs to a higher level. In addition, several additional issues arose, such as how to handle vP substances within RA, the need for new methods and approaches for the grouping of chemicals and the prediction of their toxicity, and the validation of these methods. 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 biomarkers of exposure 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

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	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.
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?	As depicted above, for the majority of the 4,000 currently used PFAS considerable data gaps exist related to current uses, exposure patterns and toxicity. Especially, human-relevant exposure and hazard data is needed for PFAS apart from PFOS and PFOA, (ii) there is a need for endpoint specific relative potency factors based on internal doses in humans, and (iii) there is a call for more intensive collaboration between toxicologists and epidemiologists to raise RAs to a higher level. In addition, several additional issues arose, such as how to handle vP substances within RA, the need for new methods and approaches for the grouping of chemicals and the prediction of their toxicity, and the validation of these methods.
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	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,

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implications for identifying vulnerable groups?	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.