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Entity	Name of person responsible	Short name institution	Date [Received]
Coordinator	Marike Kolossa-Gehring	UBA	18/12/2020
Grant Signatory	Greet Schoeters	VITO	13/11/2020

Entity	Name of person responsible	Short name institution	Date [Approved]
Pillar Leader	Greet Schoeters	VITO	13/11/2020
Work Package Leader	Greet Schoeters	VITO	13/11/2020
Task leader	Greet Schoeters	VITO	13/11/2020

Responsible author	Greet Schoeters, Rosa Lange	
Short name of institution	VITO, UBA	
Chemical group leaders	**First set of priority chemicals**	
	Phthalates/DINCH	Marike Kolossa, Rosa Lange (UBA, DE)
	Bisphenols	Robert Barouki, Elena Tarroja (Inserm, FR)
	Per-/Polyfluorinated	Maria Uhl (EAA, AT)
	Flame Retardants	Jana Klanova, Lisa Melymuk (Recetox, CZ)
	Cadmium & Chromium	Milena Horvat (JSI,SL) / Beatrice Bocca (ISS,IT)
	PAHs and air pollutants	Denis Sarigiannis (AUTH, GR)
	Anilin family: MOCA	Tiina Santonen (FIOH, FI)
	Chemical mixtures	Mirjam Luijten (RIVM, NL)
	Emerging chemicals	Greet Schoeters (VITO, BE)
	Second set of priority chemicals	
	Acrylamide	Federica Laguzzi (KI, SE)
	Aprotic solvents	Normunds Kadikis (VIAA, LV)
	Arsenic	Wojciech Wasowicz (NIOM, PL)
	Diisocyanites	Tiina Santonen and Selma Mahiout (FIOH, FI)
	Lead	Szigeti Tamás (NPHI, HU)
	Mercury	Andromachi Katsonouri-Sazeides (MOH-CY, CY);
	Mycotoxins	Paula Alvito, Maria J.Silva, Susanna Viegas (INSA, ESTeSL, PT)
	Pesticides	Helle Raun Andersen (SDU, DK)
	UV-filters	Tamar Berman (MOH-IL)

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1 Authors and Acknowledgements

Lead authors

Acrylamide	Federica Laguzzi (KI - SE)
Anilines	Tiina Santonen (FIOH - FI)
Aprotic solvents	Normunds Kadikis (VIAA, LV)
Arsenic	Wojciech Wasowicz (NIOM - PL)
Bisphenols	Robert Barouki (INSERM - FR)
Cadmium and Hexavalent Chromium	Milena Horvat (JSI,SL) / Beatrice Bocca (ISS,IT)
Diisocyanates	Tiina Santonen (FIOH - FI)
Emerging chemicals	Greet Schoeters (VITO - BE)
Flame retardants	Jana Klanova, Lisa Melymuk (MU - CZ)
Lead	Tamás Szigeti (NPHI - HU)
Mercury	Andromachi Katsonouri-Sazeides (MOH-CY - CY)
Mixtures	Mirjam Luijten (RIVM, NL)
Mycotoxins	Paula Alvito, Susana Viegas, Maria João Silva (INSA, ESTeSL-IPL – PT)
PAHs and air pollutants	Denis Sarigiannis (AUTH, GR)
Perfluorinated substances (PFAS)	Maria Uhl (EAA, AT), Þórhallur Ingi Halldórsson, Ingrid Hauzenberger, Christina Hartmann
Pesticides	Helle Raun Andersen (SDU - DK)
Phthalates & Hexamoll® DINCH®	Rosa Lange/ Marike Kolossa-Gehring, (UBA - DE)
UV filters	Tamar Berman (MOH - IL)

Contributors

Acrylamide	Agneta Åkesson, Marika Berglund, Anna Bergström, Karin Leander
Anilines	Lisbeth Knudsen, Xenia Trier
Aprotic solvents	Normunds Kadikis
Arsenic	Beata Janasik
Bisphenols	Charles Persoz, Cathrine Thomsen, Els Heyvaert, Hans Reyders, Karen Van Campenhout, Greet Schoeters, Catherine Ganzleben, Christina Hartmann, Maria Uhl, Jean-Philippe Antignac, Laurent Debrauwer, Nataša Janey Holcer, Coen Graven, Shalenie den Braver-Sewradj, Matthieu Meslin, Christophe Rousselle
Cadmium and Hexavalent Chromium	Janja Tratnik
Diisocyanates	Selma Mahiout
Emerging chemicals	J-P Antignac, G. Koppen, J. Bessems, L. Debrauwer, A. Covaci
Flame retardants	Jana Klánová, Lola Bajard, Garry Codling
Lead	/
Mercury	Mercury representatives and leaders of WP 5,7,9,10,12,13,14 leaders
Mixtures	Mirjam Luijten
Mycotoxins	Ricardo Assunção, Carla Martins
PAHs and air pollutants	Spyros Karakitsios
Perfluorinated substances (PFAS)	Xenia Trier, Tony Fletcher, Philippe Grandjean, Marqués Bueno Montserrat, Wang Zhanjun
Pesticides	/
Phthalates & Hexamoll® DINCH®	Carolin Tschersich, Nina Vogel, Kim Pack
UV filters	Zohar Barnett-Itzhaki

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2 Introduction

This deliverable reports on the achievements of HBM4EU from 2017 until 2020 per substance group. The results are reported in relation to the policy questions. The information has been compiled by the chemical group leaders and has been drafted in close exchange with the work package leaders and the substance-specific contact points for each work package. The information is based on the published deliverables and on the annual reports that have been produced by the Consortium. The information presents the steps forward that have been made by HBM4EU and serves as an input for targeted dissemination and communication of HBM4EU results to decision makers and stakeholders.

The information in the report adds to the scoping documents. The scoping documents can be found on the substance-group-specific web page of the HBM4EU web site. They contain for each substance group a review of the available evidence on hazardous properties, exposure characteristics, technical aspects, policy relevance, substance categorisation, a list of policy-related questions, and it identifies knowledge gaps and propose research activities. AD5.4 presents what has been achieved so far per substance in order to answer the addressed policy-related question. The substance-specific reports will be also published on the HBM4EU web site under the substance-specific web pages.

3 Prioritised substance group: Acrylamide

Policy Question	Short Summary of Results
1. What is the current exposure of the EU population to Acrylamide? **+** **2. Does the exposure to acrylamide differ significantly between countries and population groups? Are the main reasons for these differences related to different dietary habits or to other factors?** **+** **3. Which population groups are more at risk? Are there other sources of exposure of acrylamide that need to be discovered (e.g. smoking habits or other food sources)?**	To answer to these specific policy questions on current exposure, geographical differences and exposure determinants in the EU population, two research protocols has been developed (Task 10.4). An extensive search has been conducted to identify HBM studies performed in EU (n=25). Few studies were identified with HBM data available for acrylamide and the invitation response was low. Among them, only one study (NewGeneris) was eligible for inclusion. Determinants of the exposure have been identified in certain foods such as coffee (and solid coffee substitute), fried potato products (including potatoes and vegetables crisps), biscuits, cereals and other products such as roasted nuts, olives in brine, prunes and dates and baby food. Very few European studies have investigated other exposure determinants of acrylamide exposure in general population. No European biomonitoring studies have investigated exposure determinants of acrylamide in newborns and children. Hence, one of the proposed research protocols aims to investigate the most relevant determinants of acrylamide exposure among European adults, children and newborns and whether the exposures determinants may differ among newborns, children and adults. Also, we will investigate whether the exposure determinants may differ for dietary or non- dietary regional differences. The study will use individual data from existing European studies performed in the general population of adults and children including both sexes with sufficient representative coverage of at least one EU geographical area (North, West, East, South), available information on biomarkers of acrylamide in urine or blood and a concomitant assessment of other variables considered as possible determinants of the exposure of acrylamide. Acrylamide metabolites will be quantified in urine for children and adults and in cord blood for newborns. Individual data from NewGeneris for newborns, aligned studies for children (including GERV) and adults will be used. Each variable selected as exposure determinants will be assessed in relation to blood or urine levels of acrylamide using adequate regression models. Each analysis will be adjusted for sex, age and country. Further, a multivariable-adjusted model will be performed including in the model all the variables considered. Stratification analysis will also be performed by sex and geographical location (North, East, South and West). Currently we are waiting for the data transfer from NewGeneris and the measurements of biomarkers of acrylamide in the aligned studies. Results will allow identifying groups of people at higher risk of acrylamide exposure as well as the most relevant determinants of acrylamide exposure and hence, provide the basis for possible guidelines.

Policy Question	Short Summary of Results
4. Is there an impact from the mitigation for the production in food processing and manufacturing and REACH restrictions on the distribution of acrylamide exposure? Do we need to implement other restrictions to decrease the level of exposure of acrylamide?	Strategies for mitigation and reduction of acrylamide in food and foodstuff have been carried out since the discovery in 2000. Currently, no country has set legally binding maximum AA levels for foods. Only in 2017, the first EU regulation 2017/2158 was released with the aim to establish mitigation measures and benchmark levels for the reduction of AA in food. So far, no studies have been performed at European level to evaluate time trends of acrylamide exposure in the general population. Moreover, the awareness of acrylamide exposure has been shown to differ by European countries. Within the Task 10.4, one the research protocols aim to investigate whether the adopted measures at European level have been effective to decrease the exposure of acrylamide from 2002 up to now in the European population. Moreover, we will investigate whether these trends are equally observed in all European countries. This study will be performed using the aggregated data from published European studies with available biomarkers of acrylamide measured in urine or blood during the period 2002-2014 and aligned studies with available biomarkers of acrylamide measured in urine covering the period from 2014 up to now. We will utilise data on AA and GA concentrations in blood and urine. Published studies will have data available on biomarkers measured in urine and blood whereas the aligned studies will only have urinary biomarker data. Due to the heterogeneity in the measurements of acrylamide within participating studies, data will be harmonised to obtain comparable values. Time points will be treated as a continuous or categorical variable. Three categories of time period will be created according to the mitigation measures: before 2014, 2014-2018, and after 2018. Moreover, since the published studies have measurement of biomarkers of acrylamide before 2014 and aligned studies cover the period from 2014 and on, we will also consider to use this year as single cut-off time period. Time-line analysis for aggregated data will be performed to evaluate the relationship between time points and the mean of distribution of urine acrylamide biomarkers. Preliminary results based on published studies have shown a slightly clear increase of level of biomarkers of acrylamide for each year increase (p0.00). Findings from this study will be important to understand whether the measures adopted to lower acrylamide formation in food have been effective in lowering the levels of exposure to acrylamide in the European population.

Policy Question	Short Summary of Results
5. Are the exposure levels a concern for health? Is the exposure to acrylamide associated to cancer, neurological alterations and fetal growth in humans? Is the health risk dependent on long term or intermittent exposure to low quantity of acrylamide? **+** **6. Are the health risks dependent on age and gender?**	To answer the policy questions whether the exposure levels for acrylamide are a concern for health, specifically for cancer, neurological alteration and fetal growth, and whether the health risk is dependent on long-term or intermittent exposure to low quantities of acrylamide, activities are ongoing within WP13/WP14. To address these policy questions, Task 13.1 on mechanistic information for AOPs and 13.2 on the epidemiological evidences in relation on acrylamide and cancer, neurological and early developmental disorders and fetal growth have been implemented. 1) Acrylamide and cancer: We conducted a literature search on acrylamide and cancers including studies performed in humans, with acrylamide exposure measured through dietary assessment and/or biomarkers of acrylamide and all types of cancers as outcome (Task 13.2). A total of 65 papers were identified though Pubmed. In addition, a recent search through Scopus and Web of Science rendered 19 additional eligible papers to be included in the critical review. The extraction of the results for further interpretation from the selected papers is ongoing. Most of the epidemiological studies identified on acrylamide and cancer were performed using dietary assessment of acrylamide. There is a lack of epidemiological studies investigating the risk of acrylamide in relation to cancer using HBM studies. This preliminary observation is of importance since may explain the reason due to the epidemiological evidences have failed to show an increase risk of cancer with acrylamide exposure. Information on the AOPs leading to the development of oesophageal, gastric, and colorectal Cancer (Task 13.1) were also gathered. The molecular initiating event of the AOP involves the formation of adducts between acrylamide and its epoxide metabolite glycidamide and DNA. Acrylamide affects hormonal balances in animals, leading to increased occurrence of mammary gland tumors in rats. The main route of exposure is dietary acrylamide meaning that the gastrointestinal tract is exposed to considerable amounts of the agent; however, since the acrylamide molecule is small and hydrophilic, it reaches every organ and virtually every tissue in the body. Based on the preliminary findings: increased risk of gastrointestinal cancer may be related to intermediate levels of acrylamide rather than low levels of exposure to acrylamide and may also be related to sex differences. No conclusions could be derived on whether the current exposure levels pose a concern for health or whether age plays a role on the risk of developing oesophageal, gastric, or colorectal cancer. Also, an increased risk of oesophageal cancer (on the basis of 341 cases) emerged in subjects with intermediate levels as compared to low acrylamide intake. In relation to gender, acrylamide might be associated with colorectal cancer with specific somatic mutations, differentially in men (increased risk if activating KRAS mutation) and women (decreased risk if truncating APC mutation) (9). No conclusions could be derived on whether the current exposure levels pose a concern for health or whether age plays a role on the risk of developing oesophageal, gastric, or colorectal cancer. Stronger conclusions on acrylamide exposure and its relation to cancer in the general and potential vulnerable subgroups of the population will be produced when we integrate the further results of AOP with those of the review/meta-analysis. 2) Acrylamide and neurological alteration: Literature search has been performed on acrylamide and neurotoxicity (human cohorts, occupational, animal and in vitro studies). The final database consists of 460 studies. Among them, 22 were classified as human studies, 375 as animal studies and 63 as in vitro studies. Based on the preliminary results of the review, very few cohort studies on the association between dietary acrylamide exposure and neurological outcomes are performed, and data gap exist since no studies up to now have addressed neurodevelopmental cognitive alterations in children (only fetal growth, please see below for details).

Policy Question	Short Summary of Results
	Seventeen occupational studies have been identified in the literature search (excluding smaller case studies), showing acrylamide-related (mainly peripheral) neurotoxic alterations, but also impaired cognition. The variety of reported symptoms show involvement of the peripheral and the central nervous system, as well as the autonomic nervous system, including muscular weakness, paraesthesia, numbness in hands, feet, lower legs and arms, and unsteadiness. Acute and high exposures to acrylamide more often result in early CNS involvement, while longer exposures are associated with peripheral neuropathy. In addition, sensory impairment in the form of reduced color vision and light sensitivity has been reported and more recently impaired hearing has been suggested to be associated with acrylamide exposure. Both short term, higher exposures and prolonged lower exposures are shown to induce neurotoxicity in humans. The potential influence of age and gender with respect to acrylamide susceptibility is not clear. Differences in acrylamide metabolism may be of importance but has not been explored in relation to neurological impairments. Due to the exquisite sensitivity of the developing nervous system, a risk of early life exposure can be postulated (data gap). This concern is augmented by the likely effect of acrylamide on fetal growth (presented below). Acrylamide exposure is in some cases associated with an unhealthy diet (high fat diet) and animal experiments suggest a combined effect of acrylamide and high fat diet at least with respect to male reproductive toxicity. This may also be the case for neurodevelopmental toxicity as diet influences several of the postulated MoA/targets of acrylamide in the brain. AOP evaluation is ongoing. A proposed molecular initiating event (MIE) for peripheral neurotoxic effects of acrylamide is binding to cysteine residues in presynaptic membrane proteins. 3) Acrylamide and fetal growth: From the literature search of epidemiological studies on acrylamide exposure during pregnancy and fetal growth (Task 13.2) five publications including a recent meta-analysis based on these studies were identified and critically assessed. All of these studies used a food frequency questionnaire (FFQ) to estimate acrylamide intake and one study additionally measured hemoglobin adduct levels in umbilical cord blood. The epidemiological evidence for an inverse relationship between prenatal acrylamide exposure and reduced fetal growth is quite strong. However, with epidemiological research the question is always whether the association represents a cause and effect relationship. These preliminary findings indicate the urgent need to gather further data on the potential important effects of acrylamide on human prenatal and postnatal development. More studies along the line of the presented studies should be performed and it would also be helpful to try and interrogate the causality of the observed inverse association by investigating the biological plausibility (mechanism of action) using biomarkers of effect (such as growth factors) and susceptibility (genetic variants in acrylamide-metabolising genes). Based on the current knowledge, there are no AOP for fetal growth (Task 13.1). The construction of a new AOP on fetal growth might be complex but one can may be think to more specifically define an endpoint under the umbrella of fetal growth, such as thyroid disruption.

4 Prioritised substance group: Anilines

Policy Question	Short Summary of Results
1. What is the current occupational exposure to aniline and different aniline derivatives (including diamine forming diisocyanates) in the EU?	The available data on the occupational exposure to relevant aniline compounds have been collected under WP7.1 and summarised under AD8.1 (Report on access to occupational data). According to this analysis, the data are scattered and its coverage is limited. Many aniline compounds are nowadays restricted, which limits occupational exposure to them. Use of MOCA and technical MDA is authorised under REACH and exposure to them is rather limited in terms of number of workers. Occupational exposure to aniline itself is mostly related in its use in chemical manufacturing. Occupational exposure to anilines formed from diisocyanates, MDA/TDA as markers for diisocyanate exposure and effects for regulatory measures on the exposure to these substances, especially in small and medium sized companies, needs further data. Although some studies exist, the data is still limited. There are also some data on the occupational exposure to specific anilines (carcinogen o-toluidine and sensitiser PDA) through e.g. hair dyes but the biomonitoring data on these exposures, which may concern large number of workers, is still limited. In WP16, suspect screening is done from hairdresser's samples. This can provide additional information on the possible occupational exposure to these anilines. Results are expected during 2020-2021.
2. What is the exposure to paracetamol (aniline metabolite) among the general population?	There are single studies in Germany and Denmark on the exposure of general population to paracetamol. These has been described in aniline scoping document (D4.2 Scoping documents for 2018). To get a better overview of the paracetamol exposure, inclusion of paracetamol in the studies conducted under WP8 in general population would be needed. This is not, however, currently planned.
3. What are the risks related to these exposures?	WP5.3 deliverable report D5.1 (Human Biomonitoring in risk assessment: analysis of the current practice and 1st examples on the use of HBM in risk assessments of HBM4EU priority chemicals) describes the recent risk assessment of MOCA under REACH, which serves as a good example on the use of biomonitoring in risk assessment. In 2018 a risk assessment utilising HBM data was performed for o-toluidine under WP5, included in the Deliverable Report D5.5 (Human Biomonitoring in risk assessment: 2nd set of examples on the use of HBM in risk assessments of HBM4EU priority chemicals). In summary, a one-compartment model-based approach was used to estimate the urinary levels corresponding to the external intake levels of o-toluidine or vice versa. This allowed the comparison between available HBM data and existing binding occupational exposure level (OEL) and established cancer risk estimates. The results suggested that the workers exposed to o-toluidine have a cancer risk of 1:20 000 in the worst-case exposure scenario (0.5 mg/L in urine). The exposure levels calculated based on HBM data were below the binding occupational exposure level set under the EU Carcinogens and Mutagens Directive (BOELV, 0.44 mg/m3 corresponding to 2.2 mg/L as urinary total o-toluidine). However, the result includes several uncertainties, related especially to the limited amount of HBM data available, and therefore the RA should be seen as an example. In addition, further data on the toxicokinetics of o-toluidine in occupational settings, focusing especially to the correlations between external intake and urinary levels, would strengthen the assessment.

Policy Question	Short Summary of Results
	If o-toluidine will become authorised under REACH, HBM is recommended to be used to support exposure assessment, as regardless of the uncertainties, it is the only method able to provide information on the total internal exposure via all routes of exposure. To strengthen risk assessment of o-toluidine, PBPK modelling to calculate external intake on the basis of the urinary o-toluidine levels were performed under WP12. The results of this modelling are comparable to those obtained earlier by using urinary mass balance-based calculation approach. These results were used to calculate RCRs and were reported in D12.5. AOPs for anilines have been developed under WP13 to support human health risk assessment.
4. What is the possible impact of REACH on the exposure and risks?	WP5.3 deliverable report D5.1 (Human Biomonitoring in risk assessment: analysis of the current practice and 1st examples on the use of HBM in risk assessments of HBM4EU priority chemicals) describes the current situation with MOCA and MDA which are authorised under REACH. Because of the authorisation there are only limited number of exposed workers in EU. Occupational biomonitoring data collected under WP7.1 and summarised under AD8.1 (Report on access to occupational data) describes a decline in the exposure to MOCA observed in UK and in Finland. Therefore, MOCA was not considered as a good candidate for further research under HBM4EU although laboratories performing biomonitoring of MOCA are still needed in EU as long as it is used. Laboratories performing analysis of different aniline compounds have been listed in D9.3 (Database of candidate laboratories for the 1st prioritisation round of substances) and ICI/EQUAS for aromatic amines started in June 2019 and the results are finalised in mid-2020. Regarding MDA, AD8.1 describes the potential exposure to MDA (and similar diamine TDA) via the production and use of diisocyanates. A study to collect new data on diisocyanate and corresponding amine exposures is under preparation and samplings are planned in the end of 2020 and beginning of 2021. This new study will bring us information to study the impact of the planned REACH restriction/EU OEL for diisocyanates.

5 Prioritised substance group: Aprotic Solvents

Not all policy questions are listed – only those which have been addressed until now with notably results. All 19 PQs can be found in the scoping document.

N-methyl-2-pyrrolidone (NMP), N-ethyl-2-pyrrolidone (NEP), N,N-dimethylformamide (DMF), N,N-dimethylacetamide (DMAC)

Policy Question	Short Summary of Results
2. What is the current internal exposure of the workers in EU to reprotoxic aprotic solvents, especially with respect to female workers at reproductive age, and do they exceed Guidance values (reference and HBM values), where they are available? What data gaps exist? **3. Are there geographical differences and differences caused by industrial sector in the exposure of workers in EU to reprotoxic aprotic solvents?** **4. What is the current exposure of the general EU population to reprotoxic aprotic solvents, especially with respect to females at reproductive age as well as mothers and their young children, and do they exceed Guidance values (reference and HBM values), where they are available? What data gaps exist?**	Statistical analysis plan (SAP) for aprotic solvents is developed within WP10. The aim of the SAP is to set procedure for answering the exposure related research questions defined in the scoping document. The general part of the SAP includes statistical plans for the evaluation of time trends, geographic comparisons, evaluation of exposure determinants, a strategy for the calculation of EU reference values and a plan for conducting uncertainty analysis. It is assumed that urine (urine-spot, urine-24h, urine-morning) will be the obligatory matrix to be used for determination of chosen NMP, NEP, DMF and DMAC metabolites. Optionally a number of parameters characterising urine will be determined - total volume of urine collected, urine density of the sample, concentration of creatinine in urine of the sample, osmotic concentration of urine of the sample, specific gravity of urine (ratio of urine density compared with water density). Certain obligatory or optional variables characterising participants of the study will be applied – age, sex, education, current labour status, industrial sector of occupation, life style (frequent use of chemical household products (for cleaning, etc.) or focus on natural „ecological“ products) and consumption patterns (frequency of usage of cosmetics). Study protocols, standard operating procedures (SOPs) and guidelines, tailored and transferred questionnaires for recruitment and sampling have been elaborated within WP7 for 2th priority substances including aprotic solvents.

Policy Question	Short Summary of Results
9. Are there differences in exposure of the general EU population to regulated and non-regulated reprotoxic aprotic solvents (banned use in cosmetics)? **10. Are there differences in exposure of the workers in EU in relation to regulated and non-regulated reprotoxic aprotic solvents after the restriction for NMP will enter into force after 9 May 2020?** **11. What are differences in profiles of reprotoxic aprotic solvents observed in exposure assessment regarding occupational environment and in relation to general public taking into account spatial and temporal distribution?**	
13. What are the best indicator`s substances (markers) to identify hazardous exposures to aprotic solvents as a whole? **14. What are the analytical options available with respect to aprotic solvents (gas chromatography-mass spectrometry versus liquid chromatography-tandem mass spectrometry for biological matrices, other methods in addition, methods for environmental media)?**	In WP9 a suggested list with most suitable biomarkers, matrices and analytical methods has been elaborated based on published data. 5-hydroxy-N-methyl-2-pyrrolidone (5-HNMP) and 2-hydroxy-N-methylsuccinimide (2-HMSI) are specific biomarkers of NMP for analysing urine samples and sufficiently low detection limits can be achieved. 5-hydroxy-N-ethyl-2-pyrrolidone (5-HNEP) and 2-hydroxy-N-ethylsuccinimide (2-HESI) are specific biomarkers of NEP exposure and are suitable for the monitoring of non-occupationally exposed populations. For both NMP and NEP GC-MS or GC-MS/MS analyses reached the lowest LODs and can currently be regarded the methods of choice. Urine is the preferred matrix for exposure characterisation for both NMP and NEP. Further need for method development in relation to NEP metabolites is identified due to scarcity of published data on NEP. The following exposure biomarkers have been described for DMF: DMF in urine, N-methylformamide (NMF) in urine, N-acetyl-S-(N-methylcarbamoyl)cysteine (AMCC) in urine, 3-methyl-5-isopropylhydantoin hemoglobin adducts (NMVal/NMHb) in red blood cells. NMF in urine is the preferred option for occupational exposure by application of GC-NPD analytical method, however, it seems unfit to capture environmental background exposure levels, AMCC in urine seems to be the best option for exposure assessment in the general population but the sensitivity of the method should be improved. AMCC is usually measured by LC-MS/MS.

Policy Question	Short Summary of Results
	It shall be taken into account that AMCC may also be formed from the dietary uptake of methyl-isocyanate which is a component of wine and cruciferous vegetables such as cabbage, turnips and cress. Therefore, AMCC may lack specificity to reflect the environmental exposure of DMF. The haemoglobin adducts might serve as indicators of longer term exposure, however their use in biomonitoring seems questionable due to complicated sample (isolated globin) preparation. The following exposure biomarkers have been identified for DMAC: the DMAC itself, N-methylacetamide (NMAC), N-hydroxymethyl-N-methylacetamide (DMAC-OH) and S-(acetamidomethyl) mercapturic acid (AMMA). Nevertheless, methods for the quantification of DMAC metabolites in human matrices are not well established. Urine seems to be the best matrix for analyses of DMAC metabolites. The DMAC biomarkers have been measured by GC-MS, GC-NPD, GC-FPD, LC-MS or UHPLC-MS/MS. UHPLC-MS/MS gave the best sensitivity for all four biomarkers. DMAC-OH is known to be converted to NMAC under high temperatures in the GC injector, but this problem can be avoided by LC analysis. The detection and quantification of DMAC metabolites needs further method improvement in order to enhance the sensitivity for low exposed general population. Institute for Prevention and Occupational Medicine of the German Social Accident Insurance (IPA) has held an advanced training course on method improvement for NMP and NEP on 23rd November 2018 in in Bochum, Germany (the 2nd HBM4EU training school). In total, 14 candidate laboratories for the analysis of aprotic solvents have been identified in 7 countries.

6 Prioritised substance group: Arsenic

Policy Question	Short Summary of Results
1. What is the current exposure of the EU population to arsenic?	When reviewing the literature on exposure to inorganic arsenic, recent review data indicate existing drinking water pollution in Europe and possible exposure to iAs. There are several such regions in Europe where the arsenic content of water is between 7 and 90,000 mcg/l [Medunic et al 2020]. In addition to drinking water, the European population is exposed to arsenic through the consumption of food products containing, in addition to organic arsenic, inorganic arsenic. Within the framework of WP 7 activities, the identification of studies related to biological monitoring of exposure to As. A protocol has been developed under the activities of WP 10 (Task 10.4) on the possibility of using available studies related to the As exposure. From the available data (IPCHEM), 24 projects (studies) on the adult, adolescent, child and pregnant women populations were found. Arsenic was analysed in different matrices (cord blood, blood, urine, breast milk), as total arsenic and/or chemical forms. A thorough exposure assessment should be carried out based on the data made available by stakeholders (WP 10) and as part of the compensatory studies carried out for the activities of WP 8 (Task 8.1). Classification (QA/QC) of laboratories carrying out analyses of arsenic and chemical forms in urine (WP 9) has been carried out as this form of exposure assessment was considered the most appropriate. A list of approved laboratories is currently available and includes 3 laboratories. The results obtained from standardised testing will also enable European Reference Values (ERVs) to be obtained under task 10.3.
2. What biomonitoring and exposure (environmental and occupational) data on arsenic, relevant to the European population, are currently available.	The activities carried out in WP 7 and WP 10 provide highly differentiated data based on the IPCHEM database for the European adult, child, adolescent and pregnant women population. Compensatory research (WP 8) offers the possibility of obtaining analytically homogeneous (single matrix, most favourable biomarker) data on the population of children, adults and adolescents
3. What is the geographic spread of the current exposure and how does it relate to different exposure sources (environmental; dietary sources)?	In Europe there are several regions of so-called hotspots where there is significant exposure to inorganic arsenic with drinking water (e.g. Romania, Hungary, Slovakia). In Poland, the population exposed to iAs (air, soil) occurs as a result of industrial activities. General population in Finland,Greece, Italy, Czech Republic is also exposed to arsenic compounds. In other European countries, where the concentration in drinking water is below the WHO recommended value of 10 mcg/l, we have to do with exposure related to the consumption of food containing inorganic arsenic. A recent investigation by EFSA (European Food Safety Authority) found that dietary exposure to inorganic arsenic in Europe is not as high as was previously assumed. There are currently no recommended maximum levels of inorganic arsenic in food at EU level, however, EU maximum limits for inorganic arsenic (particularly in rice and rice products) are being discussed. The analysis of exposure in the European population is ongoing. Once the required data from the available datasets will be acquired, the data will be compared statistically and visualised with respect to geographic regions (north, south, east, west), countries and the NUTS regions.

Policy Question	Short Summary of Results
	In cases of individual data (which is the majority of the datasets), we'll be able to confound for the known and hypothetical determinants of As exposure (e.g. diets) to reveal the geographical and/or environmental pattern(s). This will also allow as to identify the main sources for possible exposure.
4. Which population groups are most at risk?	It is believed that risk groups for arsenic exposure are populations consuming high arsenic water and infants and children fed on rice-based foods. Long-term exposure to arsenic from drinking-water and food can cause cancer and skin lesions. It has also been associated with cardiovascular disease and diabetes. In utero and early childhood exposure has been linked to negative impacts on cognitive development and increased deaths in young adults. Children's metabolism of iAs differs from that in adults, which might explain the lack of data on arsenic metabolism as a susceptibility factor for arsenic toxicity in children. Children had higher arsenic methylation efficiency than adults, and there was no difference between boys and girls. Studies in vulnerable populations and studies for a better understanding of the health effects of inorganic arsenic in the population at exposure levels in EU are greatly needed. Aligned study under WP 8 should fill in the missing data, including for the most vulnerable population, namely children. The RV 95 for total arsenic in urine, according to the findings of the German HBM survey, is 15 µg/L for children and adults who did not eat fish during 48 hours prior to sample collection [Schulz et al., 2011]. The GM levels of total arsenic in European populations were from 0.5 µg/L to 1 µg/L in blood and from 4µg/g to 16 µg/g creatinine in urine. There was no obvious difference observed between children/adolescents and adults [WHO 2015].
5. What factors (genetic polymorphisms) make people more susceptible or not to the risk of health effects due to arsenic exposure? How are the best and more sensitive biomarkers for identification of reliable arsenic exposure and to link to potential adverse health-effect?	Many works indicate the relationship between the genetic polymorphism of arsenic-metabolising enzymes and the efficiency of methylation processes. The existence of such relationships is confirmed by the works of Gonzales-Martinez et al (2020) and Kazenifar et al (2020). There are several potential biomarkers for arsenic exposures. Preferred biomarkers are determination of As and its chemical forms in urine. Non-invasive, ease collection and because the majority of absorbed arsenic and its metabolites is eliminated via urine puts this type of markings in a privileged position. WP 13 (Task 13.2) and WP14 groups have coordinated the selection of biomarkers of effect according to their utility in human studies, the identification of needs for the implementation of both classical and novel biomarkers of effect and the decision criteria for their validation. As part of the assessment of potential effects of various chemical compounds, including arsenic, human health, a publication was prepared entitled "Arsenic and human health. "Scoping review - the association between asthma and environmental chemicals".
6. What are possible health effects resulting from chronic low exposure to arsenic from food consumption?	Low-level groundwater As contamination (≤50 µg/L) on public health by identifying the varied health effects, e.g., disorders of the skin, lungs, cardiovascular system, endocrine dysfunction, neuropsychological complications, aberrant pregnancy outcomes, liver and skin ailments, risk of carcinogenesis and mortality. In vitro and in vivo studies related with low-level As, depicted interplay of genomic variants, DNA damage and repair, aberrant methylation, inflammation, immune suppression and deregulation of signal transduction, which might have influenced health complications, including risk of carcinogenesis.

Policy Question	Short Summary of Results
	In addition to the tasks carried out under WP 12 and WP 13, in search of the interdependencies between the exposure and health effects and new specific effect biomarkers, in task 12.3, parameterisation of the PBTK model was performed. The PBTK models are increasingly being used as an effective tool for designing toxicological experiments and for performing extrapolations necessary for risk assessment. Published human PBTK models have been reviewed, with particular emphasis on the values of pharmacokinetic parameters and methods used to estimate these values. In most of the models studied, the preferred approach to parameter value estimation was to use literature data based on experimental data (in vivo or in vitro). In the absence of experimental data, quantitative structure activity relationships (QSAR) were used for parameterisation. The work performed within this task was presented in "AD12.10 - Report on parameterisation of the second set of priority substance"
7. What are the best analytical methods should allow for differentiating species in urine?	The determination of arsenic in biological specimens requires sensitive analytical methods, performed under good quality control conditions. Due to the possibility of separating the different chemical forms that are relevant in the toxicity assessment, it was considered that the assessment of the different forms alongside total arsenic would be the most advantageous biomarker in the exposure assessment. The use of the ICP-MS technique in combination with separation techniques, e.g. HPLC, now appears to be the most advantageous analytical technique to use in the As exposure assessment. Under WP9 activities, laboratories invited to participate in proficiency testing were qualified on the basis of QA/QC checks carried out. Currently, the proficiency tests are completed, 3 laboratories that have successfully passed the controls are included in the list of laboratories performing the tests in the assessment of the second list of priority substances.
8. How can harmonised, validated and comparable information be collected to support and evaluate current policies?	Analysing the data on arsenic exposure in different age groups, one can see a lack of such data for a group of adolescents. The alignment study plans to assess exposure to different forms of arsenic (speciation) as the most reliable biomarkers.
9. How can HBM4EU results support European policy decisions?	The results of the project will identify stakeholder groups, prioritise the assessment of exposure to chemicals and meet the needs for biological monitoring research for stakeholders, starting with policy makers and researchers

7 Prioritised substance group: Bisphenols

Policy Question	Short Summary of Results
1. What is the current exposure of the EU population to BPA, BPS and BPF?	Analytical aspects: As a preliminary step to be able to address this policy question, in WP9 a prioritised list with most suitable biomarkers, matrices and analytical methods has been elaborated (see D9.1). The biomarkers of exposure, matrices, analytical methods and method detection limit (MDL) selected for bisphenols are listed below: - Bisphenol A: BPA, Urine (0.5 mL), LC-MS-MS, MDL: 0.02 ng/mL - Bisphenol S: BPS, Urine (NA), LC-MS-MS, MDL: 0.03 ng/mL - Bisphenol F: BPF, Urine (NA), LC-MS-MS, MDL: 0.06 ng/mL For BPA, BPS and BPF urine should be preferred as the matrix of choice for exposure assessment in the general population. The risk of contamination of the samples during the sampling/preparation procedure should be monitored by field and laboratory blank controls. Now the procedure for evaluating and managing this possible external contamination level remains to be harmonised between laboratories as a prerequisite for reliable inter-country data comparison and analysis. Commercially available internal standards and biomarkers for BPS, BPF and other BP's are not yet well implemented in all laboratories proposing bisphenol analyses. More broadly, for BPS, BPF and other BP's there is a general lack of peer-reviewed, published methods, especially in urine, and experience in HBM studies is less well established as compared to BPA. Up to now quantifiable measures in urine with quality assured (labelled) internal standards for BPS and BPF are not available in Europe. The interlaboratory assays (ICI/EQUAS) organised within WP9 in 2018/19 permitted to have a better picture of the current situation on these analytical aspects and of the real existing capabilities for bisphenol analyses in the different laboratories proposing these analyses. After 3 rounds of ICI/EQUAS, 24 laboratories have been qualified to analyse bisphenol A in HBM4EU (AD9.3). Chemical analyses of BPS and BPF have been improved and a sufficient number of laboratories was achieved after the 3rd ICI/EQUAS round (18 labs BPS and 13 labs BPF). Additional questions in regard to natural occurrences of BPF in mustard (Zoller et al., 2016)1 need to be answered in order to ensure the specificity of this biomarker to represent non-natural exposures to BPF. A more detailed investigation of the isoforms of BPF (4,4'-BPF, 2,2'-BPF and 2,4'-BPF) in terms of exposure biomarker validity might be warranted (D9.7). WP16 is also contributing to refine and/or increase the knowledge regarding the relative proportions of free versus conjugated forms of these contaminants. This aspect is of major importance in consolidated exposure assessment, characterised toxicological impact, and contribution to PBPK modelling for exposure-health studies. It is also important to address the issue of contamination during sample preparation mentioned above. What do we learn from previous surveys? WP5 has prepared a scoping paper on the development of Human Biomonitoring (HBM) indicators on chemical exposure in the European population. A case study on HBM indicators from DEMOCOPHES data has been reported for BPA (see D5.3).

[1] Zoller O, Brüschweiler BJ, Magnin R et al. 2016. Natural occurrence of bisphenol F in mustard. Food Addit. Contam. Part A, Chem. Anal. Control. Expo. Risk Assess. 33:137-146. doi: 10.1080/19440049.2015.1110623.

Policy Question	Short Summary of Results
	This led to the following results (exposure levels in the population): in children, the weighted geometric mean (95% CI) for urinary BPA equaled 1.97 µg/L (1.81-2.15) in the total European study group. In mothers, it equaled 1.78 µg/L (1.62-1.94), suggesting a tendency for higher levels in children compared to their mothers. AD 5.3 reports that HBM based result indicators can provide information relevant to address current exposure of EU population. However, in IPCHEM/repository, there is not enough data available to answer this now (only 4 aggregated data collections on BPA of 3 different countries). Survey planning and harmonisation. In 2018 WP8 has establish a sampling frame for Europe to align ongoing/planned studies to collect HBM data of the prioritised chemicals with EU wide coverage. Due to the scarce availability of recent exposure data on the first set of priority chemicals and due to financial limitations, it was decided to focus on specific chemicals per age group to achieve EU wide coverage of these chemicals within the selected age groups. Therefore, bisphenols (A,S, F) will be analysed in adults (20-39 years) (D8.4). Samples are collected in 11 different European countries. In 2019 specific questionnaires on bisphenols for children and for adolescents where developed under T7.3, available for download at the online library. Statistical plans. WP10 has elaborated, among others, a specific statistic data analysis plan for bisphenols. The plan defines all the necessary variables for the statistical analysis to address bisphenol specific research questions on general exposure levels, time trends, geographic comparisons and exposure determinants (see D10.2).
2. Do different regulatory controls across the EU concerning in particular BPA lead to different exposures?	As a very preliminary step to be able to address this policy question, WP7 has elaborated a study protocol for harmonised recruitment and sampling (see D7.3). This procedure provides the main essential points for the planning of new studies or if an existing study is to be aligned or biobanked samples are to be used in the frame of HBM4EU. A specific questionnaire for bisphenols has been designed to collect all the necessary information concerning individual characteristics of the participants (sociodemographic, dietary, occupational, lifestyle, environmental and health factors). The outcome of these questionnaires might contribute to find out whether there are HBM data or suitable samples available before and after the ban in France, Sweden and Denmark. As an action to guarantee the comparability and the quality of the analytical results within HBM4EU, WP9 is implementing a complete tailor-made ICI/EQUAS program covering until now 74 parameters, including the analysis of BPA, BPF and BPS in urine. The 33 candidate laboratories identified in task 9.2 were invited to join the 1st ICI round and about the 72% of them have participated (different number of laboratories for the different bisphenols). The 1st round finished in July 2018 and the 2nd ICI round at the end of 2018. A 3rd ICI/EQUAS took place and based on the combined results of the ICI/EQUAS scheme 24 laboratories have been qualified to analyse bisphenols in HBM4EU (AD9.3). WP5 is contributing to guide decision makers for using HBM data in a broad sense (awareness raising, remediating measures, and improved regulation), trajectories towards participatory processes have been initiated. A first case study on policy uptake of HBM results has focused on bisphenols and phthalates (see D 5.4). The bisphenols case (mainly focusing on BPA) is characterised by a persistent controversy, fuelled by the discrepancy between standardised regulatory studies (used for formal risk assessments) that do not report health effects, and an increasing number of academic studies reporting effects at current exposure levels (low doses), but lacking reproducibility and therefore not meeting the quality standards for regulatory risk assessment. A first goal of HBM4EU for the bisphenols is therefore to guarantee quality controlled HBM data measurements via interlaboratory assays (ICI/EQUAS) organised within WP9.

Policy Question	Short Summary of Results
3. Are bisphenols exposure levels of concern for health?	Establishing Health-based Guidance values. For BPA, there are different HBM-HBGVs (Human Biomonitoring derived Health-Based Guidance Values): 1) the German HBM-I-value for children and the German HBM-I-value for adults2 2) the BE value based on the one hand on the pTDI from Health Canada and on the other the BE value based on the US EPA RfD and EFSA TDI3. The German HBM-I value was used here as primary health-based guidance value in the absence of an HBM HBGV derived in HBM4EU. The use of the German HBM-I-value indicated that in <5% of the Danish children and in <5% of the mother-child pairs in Belgium the measured urinary BPA concentrations exceeded the German HBM-I-guidance value. Exceeding the HBM-I-value implies that the occurrence of a certain health risk cannot be excluded with sufficient certainty. As the percentage of the population exceeding the HBM HBGV was <5% in Danish mothers, Belgian children and mothers, the extent of exceedance indicator, based here on the ratio of the 95% percentile over the HBM-I-guidance value, was <1. None of the participants in the six European countries exceeded the BE values for urinary BPA which are older and higher than the HBM-I. The BE value corresponding to the oral provisional tolerable daily intake (pTDI) of 25 μg/kg-d from Health Canada is 1 mg/L (1.3 mg/g creatinine); value corresponding to the US EPA reference dose (RfD) and EFSA tolerable daily intake (TDI) estimates (both of which are equal to 50 μg/kg-d) is 2 mg/L (2.6 mg/g creatinine). Towards the end of 2018, in WP5, ANSES and UBA started working on the establishment of new HBM4EU health-based guidance values for BPA both in the general population and in occupational settings. These values have been reported in D5.14 and are detailed below under policy question 8. Selecting effect markers. A strategy for the selection of effect biomarkers for their potential implementation in HBM4EU aligned studies has been presented and exemplified in three case studies in relation to the bisphenols family of compounds. Because bisphenols have complex mode of action (MoA), implementation of effect biomarkers at different levels of biological organisation (e.g., DNA, RNA, proteins or metabolites) seems necessary. The suitability of using effect biomarkers from the WP14 inventory (D14.3) to represent possible AOPs identified by WP13 has been explored for bisphenol A and female reproductive health, glucose homeostasis and neurological effects. The technical and scientific limitations have also been discussed (AD14.3). A comprehensive review of the literature was performed, creating the first inventory of effect biomarkers for bisphenols. Several epigenetics, gene transcription, oxidative stress, reproductive, glucocorticoid and thyroid hormones, metabolic and allergy/immune biomarkers were first studied, and then, promising effect biomarkers related to altered neurodevelopment and reproductive outcomes including brain-derived neurotrophic factor (BDNF), kisspeptin (KiSS), and gene expression of nuclear receptors were prioritised, providing mechanistic insights based on in vitro, animal studies and AOP information. Finally, the potential of omics technologies for biomarker discovery and its implications for risk assessment were also discussed (Mustieles et al., 2020)4. AOPs and BPA. WP13 has produced a report on adverse outcome pathways (AOPs) for the first set of prioritised substances within HBM4EU. However, most of the effort was devoted to BPA substituents (see below). PBTK. Within task 12.3, biological half-lives (t1/2) in human have been compiled that contribute to the refinements of the PBTK models and estimation of internal doses for 1st set of priority compounds. The t1/2 values (hours) compiled for bisphenols [BPA and its substitutes (BPS, BPF)] in blood/serum or urine were generally low, with median values lower than 7 h (D12.3).

[2] German Human Biomonitoring Commission, 2012. Stoffmonographie bisphenol-A (BPA) – Referenz- und Human-Biomonitoring-(HBM)-Werte für BPA im Urin. Bundesgesundheitsblatt, Gesundheitsforschung, Gesundheitsschutz 55 (9), 1215-1231.

[3] Krishnan, K., Gagne, M., Nong, A., Aylward, L.L., Hays, S.M., 2010. Biomonitoring Equivalents for bisphenol A (BPA). Regulatory toxicology and pharmacology: RTP 58, 18-24.

[4] Mustieles V, D'Cruz SC, Couderq S, Rodríguez-Carrillo A, Fini J-B, Hofer T, Steffensen IL, Dirven H, Barouki R, Olea N, Fernández MF, David A. Bisphenol A and its analogues: a comprehensive review to identify and prioritize effect biomarkers for human biomonitoring. Environment International. 2020. In Press.

Policy Question	Short Summary of Results
	WP12 has also assessed exposure pathways leading to internal dose using a combination of external exposure determinants/modifiers and, in the case of bisphenol A, a dedicated model was developed by WP12 partners. Furthermore, tissue levels were determined based on blood levels and blood/tissue partition coefficient. For bisphenols, the BPA exposure of the Elfe study pregnant women was estimated individually from the HBM data (here, the urinary concentration of total BPA) using a reverse dosimetry approach based on PBPK modelling. The aim was to estimate the internal exposure in the mothers and their foetuses. Two scenarios of exposure corresponding to the same amount of administered BPA over the pregnancy were tested. The scenario I assumes a constant and continuous exposure to BPA via ingestion and dermal contact over the whole pregnancy. The scenario II models three diet intakes and 2 dermal intakes by PCPs each day of the pregnancy. Our modelling results showed that the urinary and plasma concentrations in mothers evolves over the day according to the BPA intakes (3 meals and 2 dermal contacts). On the contrary, the foetal plasma concentration is quite stable due to transfer rates that are quite low within a day. The maternal and foetal plasma concentrations were of the same order. Available HBM data for Europe on bisphenols have been reviewed, as well as the regulatory thresholds available for bisphenols. Using toxicokinetic modelling, environmental (i.e., non-occupational) exposure to bisphenols has been estimated for the reviewed HBM data. The uncertainty interval of the external exposure ranged from 0.01 to <1 µg/kg bw/day for the different European cohorts. All reconstructed exposures were below the regulatory threshold selected, i.e. the temporary tolerable daily intake set by EFSA in 2015 (4 µg/kg/day). Thus, the obtained risk characterisation ratios from the 95th percentile of the population exposure were below 1, indicating that no risks were associated with BPA exposure in the studied European cohorts.
4. Is occupational exposure of cashiers a health concern?	WP5 has elaborated a concept document on the strategy for the derivation of health-based guidance values for the general population and for occupationally exposed adults (see D5.1). In the case of occupational BPA exposure of cashiers, HBM data can be used to support modelling data, giving a stronger basis for the assessment. However, BPA HBM based risk assessment included some uncertainties related for example on the fraction of free BPA available for systemic distribution after dermal exposure. As of January 2020, BPA in thermal paper is restricted because of health risks for pregnant workers and consumers exposed to it in thermal paper (France, 2014). The analyses of biomonitoring studies performed by ANSES and by EFSA (EFSA, 2015) were included into an updated version of the restriction dossier. A draft on the derivation of Human Biomonitoring Guidance Values (HBM-GV) for BPA is currently under development. HBM-GV will help interpretation of the potential health impact of internal chemical exposures measured in workers and in the general population through HBM. At this stage, urinary BPA concentration distributions have been reconstructed based on published EU HBM studies for further comparison with the derived HBM-GV on BPA. Information on BPA HBM data in occupational settings has already been collected.
5. What is the toxicity of BPA substitutes and are current exposure level of concern?	Computational Tool development. In order to assess the putative toxicological impact of BPS and BPF, new tools were developed. These tools are based on text mining using artificial intelligence as well as on systems biology tools (Carvalho et al, EHP, 2019)5. Using different combinations of these tools it was possible to identify the most likely toxic outcomes of exposure to these substituents.

[5] Carvaillo, J-C., Barouki, R., Coumoul, X., Audouze, K., 2019. Linking bisphenol S as an environmental chemical stressor to key events and adverse outcomes using a text mining-based computational approach. EHP- Environmental Health Perspectives. https://doi.org/10.1289/EHP4200

Policy Question	Short Summary of Results
	Putative toxic effects of BPA substituents. Using the tools mentioned above it was possible to highlight obesity as one of the major potential health endpoint of BPS which was related to the biological activity of adipogenesis. The characteristic key events were decreased lipolysis, increased adipocyte formation, fatty acid uptake and lipogenesis. These are initiated by the disruption of the activity of 1) several transcription factors including estrogen receptors or ERR gamma, 2) enzymes such as hormone-sensitive lipase, or 3) expression of adipogenic biomarkers including PGC-1 alpha & perilipin 4 (D13.4). Using refined computational tools applied to bisphenol F allowed us to link BPF stressor to an AOP network for thyroid cancer (Rugard et al. 2020)6 A research study on (Bishenols A, S, and F) male fertility endpoints and endocrine disruptive effects in cohort studies is currently ongoing (AD13.3). To further investigate the potential of seminal plasma as a key resource for study of male exposure to environmental pollutants and effect on general health, bisphenols will be measured in the seminal plasma of sub-fertile (case) and fertile (control) males. More than 150 samples (75 pairs) have been collected, characterised and stored. Quantification of bisphenols in all samples is expected to be completed by end 2020 and comparative analysis between fertile and sub-fertile males undertaken early 2021.
6. Are health risks age and gender dependent?	WP7 has run a NHCPs online consultation on existing HBM surveys. The outcome from 124 questionnaires has been analysed in D7.1. For bisphenols, an analysis by European-defined region showed that the identified studies included predominantly the North (Denmark, Estonia. Finland, Iceland, Ireland, Latvia, Lithuania, Norway, Sweden, UK) and the West (Austria, Belgium, France, Germany, Luxembourg, The Netherlands, Switzerland). For the studies reported as having a national representativeness level, the majority involved children and newborns. In studies with children, bisphenols were among one of the most analysed substances. As previously described above, in WP12, the BPA exposure of the Elfe pregnant women was estimated individually from the HBM data (here, the urinary concentration of total BPA) using a reverse dosimetry approach based on PBPK modelling. Two scenarios of exposure corresponding to the same amount of administered BPA over the pregnancy were tested. (Detailed results in D12.4)
7. Can we find evidence for low-dose effects within mixtures?	During 2018 WP13 addressed exploration of available cohort data for bisphenols and (neuro) developmental and reproductive outcomes (D13.3). Further studies were outlined addressing mixture effects of PFAS, bisphenols and/or phthalates in children.

[6] Rugard M., Coumoul X., Carvaillo J-C., Barouki R., Audouze K. (2020) Deciphering Adverse Outcome Pathway Network Linked to Bisphenol F Using Text Mining and Systems Toxicology Approaches, Toxicological Sciences, 173 (1), 32–40, https://doi.org/10.1093/toxsci/kfz214

<table>
<tr><th>Policy Question</th><th>Short Summary of Results</th></tr>
<tr><td>8. How can HBM feed into assessment of the Tolerable Daily Intake (TDI) for BPA, as set by the European Food Safety Authority (EFSA)?</td><td>
WP12 has developed a roadmap for PBTK/TD model refinement and analysis for priority substances including bisphenols (see AD12.2)
This PBPK model can reproduce the BPA chemical-specific pharmacokinetic data for oral exposure though solid form (cookie) and is reliable with regard to its predictions of BPA in serum (Thayer et al 2015, N=3 volunteers), BPAG in serum (Thayer et al. 2015, N=3 volunteers), cumulative excretion of BPAG in urine (Thayer et al 2015, N=3 volunteers and Volkel et al. 2002, 2005).
For oral exposure though liquid form (soup), the PBPK model has been revised (re-calibrated by optimisation of the oral uptake constant) however not evaluated with new data.
The model should be further evaluated, in particular towards the biological relevance of modelling the enterohepatic recirculation.
WP12 has optimised the methodology for exposure reconstruction (AD12.6), which was applied in available HBM data.
For bisphenols, in particular BPA: the EU population was estimated to have an average daily intake of 0.05 µg/kg_bw•d, a value that is much lower than the corresponding temporary tolerable daily intake set by the European Food Safety Authority (EFSA). Even the highest level of HBM measurements (corresponding to Italian adult population) corresponded to a daily intake of 0.77 µg/kg_bw•d, still significantly lower than the EFSA t-TDI. HBM data for different age groups including young children, adults and pregnant mothers were used for the assessment. Both a life course PBTK model and a pregnancy PBTK model were used to allow for more accurate reconstruction of external exposure taking note of the physiological and metabolic differences characteristic of different age windows during the life course and in utero.(AD12.5). In WP5, ANSES and UBA have worked on the derivation of HBM-GVs for BPA both in the general population and in occupational settings. (D5.14).
The table below shows the derived boundary values for total BPA in urine consistent with the concentration of free BPA in plasma after 100% oral exposure to the t-TDI of 4 µg/kg bw/d.
<table>
<tr><th>Population group</th><th>High boundary value for total BPA in urine (100% oral exposure to BPA) → HBM-GVGenPop</th><th>Corresponding concentration of free BPA in plasma</th></tr>
<tr><td>Adult (45 years, 70 kg)</td><td>233 µg/L</td><td>6.9.10-3 µg/L</td></tr>
<tr><td>Child (5 years, 19 kg)</td><td>137 µg/L</td><td>13.7.10-3 µg/L</td></tr>
</table>
Despite the fact that inhalation seems to be the most important route of BPA exposure for workers, data characterising the toxicokinetic of BPA after inhalation in humans is lacking. It is presumed that a fraction of inhaled BPA is actually absorbed by the oral route and is thus subject to first-pass hepatic metabolism. However, due to the paucity of kinetic data regarding the inhalation route and the fact that all existing OELs are based on non-systemic respiratory effects, it is not possible to derive a HBM-GVworker based on atmospheric levels to BPA likely to induce toxic effects at the workplace.
Taking advantage of the modified PBPK model from Karrer et al. (2018)7, which includes the oral and dermal route of exposure, the concentration of total BPA in urine after dermal exposure to BPA was rather estimated based on the plasmatic concentration of free BPA (e.g. the toxicologically-relevant chemical form) generated after 24h average oral exposure to the oral DNEL for workers of 8 µg/kg bw
</td></tr>
</table>

[7] Karrer C, Roiss T, von Goetz N, Gramec Skledar D, Peterlin Mašič L, Hungerbühler K., 2018. Physiologically Based Pharmacokinetic (PBPK) Modeling of the Bisphenols BPA, BPS, BPF, and BPAF with New Experimental Metabolic Parameters: Comparing the Pharmacokinetic Behavior of BPA with Its Substitutes. Environ Health Perspect.;126(7):077002. doi:10.1289/EHP2739

Policy Question	Short Summary of Results
	Regarding BPA exposure at the workplace, the level of urinary total BPA was estimated after the dermal uptake of BPA which would generate the same free BPA concentration in plasma (considered as the bioactive form) as a 24h-averaged intake to the ECHA's DNEL for oral uptake of 8 µg/kg bw for workers. The estimated concentration of urinary total BPA is equivalent to, or exceeds the 95th percentile of total BPA in urine measured in different European HBM studies conducted in the general population. Thus, no HBM-GVworker was proposed, as the high background level of BPA coming from environmental exposure - mostly through food intake - is making the discrimination with the occupational exposure to BPA difficult
9. Is it important to eliminate legacy BPA from material cycles (i.e. waste till receipt rolls) when implementing a circular economy in order to protect human health?	Based on the studies carried out in HBM4EU but also in several other projects that were analysed by HBM4EU, it is possible to state that legacy can possibly have health impact. However, this will be better assessed when Guidance values will be obtained for BPs.

8 Prioritised substance group: Cadmium (Cd)

Policy Question	Short Summary of Results
1. What is the current exposure of the European population to Cd?	Inventory of studies holding Cd exposure data was obtained through WP7 (task 7.1) with an online questionnaire which was distributed with an aim to identify existing HBM studies. Within the WP10 (task 10.4) a substance-specific research protocol has been elaborated to exploit the available datasets with an aim to assess current Cd exposure of the European population and its geographical distribution. From the datasets having information on Cd internal exposure, 37 datasets from 17 countries have so far confirmed sharing of individual or aggregated data (the majority is individual data, 33 datasets) to assess the exposure in Europe and its geographical variability. Based on the data available, we decided to look at the exposure data for the period between 2007-2017. However, the work is in progress (acquisition of individual or aggregated data from data providers) and the number of datasets is constantly being updated. Preliminary assessment of the data available from the above-mentioned datasets has been done. So far, we have 27 datasets having Cd measurements for adult general population from all 4 geographic regions (north, south, east, west). Although the preferred matrix for internal Cd assessment is Cd in blood, the majority of the datasets (23) have the measurements available for urine, while only 10 for whole blood. Additionally, we have 5 datasets for Cd in cord blood, 3 datasets for Cd in child's blood, 2 for Cd in adolescent's blood, and 10 for Cd in child's urine and 1 for Cd in adolescent's urine. Based on the concentration ranges reported for adults, the levels in urine span from below LOD to 5.34 µg/L and in blood from below LOD to 6.26 µg/L. In cord blood the levels are <LOD-2.5 µg/L, while in children/adolescents <LOD-22.9 µg/L and <LOD-0.144 µg/L in blood and urine, respectively. However, the mean values for all datasets that have this data available are all below the established HBM I value of 1 µg/L urine (adults) and 0.5 µg/L urine (children/adolescents). Further assessment as described in the research protocol is on-going. Additional exposure assessment will be performed based using harmonized methodology developed and agreed in task 8.1 (aligned studies) to obtain EU-wide coverage for recent exposure (2014-2018). Cadmium will be measured in samples of identified on-going studies (200-300 participants per study). The studies selected include adults (20-39 years) from 8 countries distributed among 4 geographical areas of Europe: Denmark, Iceland, Czech Republic, Poland, Croatia, France, Switzerland, and Germany. Cadmium will be determined in urine (available in all 8 studies) or whole blood (available in 3 studies). Among others, results obtained in aligned studies will allow further evaluation of the proper use of biomarkers (urine vs. blood) at low level of exposure. The laboratories performing laboratory analysis have been tested through the QA/QC scheme, which has completed the third round of proficiency tests for the determination of Cd in urine and whole blood. The first list of approved laboratories is now available and includes 33 labs for urine samples and 22 labs for Cd in blood. The comparable results obtained from the aligned studies will also enable derivation of European Reference Values (ERVs) as part of task 10.3.

Policy Question	Short Summary of Results
2. Does the exposure to Cd differ significantly between countries and population groups? What are the main reasons for differences in exposure?	This question will be answered once the work described in the substance-specific research protocol developed within task 10.4 is completed. The work is in progress as described above. Spatial analysis will be done according to the Statistical analysis plan (Deliverable 10.5, Section 7). Once the required data (individual or aggregated) from the available datasets will be acquired, the data will be compared statistically and visualized with respect to geographic regions (north, south, east, west), countries and the NUTS regions. In cases of individual data (which is the majority of the datasets), we'll be able to confound for the known and hypothetical determinants of Cd exposure (e.g. smoking) to reveal the geographical and/or environmental pattern(s). This will also allow as to identify the main reasons for possible differences.
3. Is there a significant time trend of Cd levels in existing population studies?	Only 3 datasets have been identified that have repeated Cd measurements available: German ESB and GerES (from 1986), Czech Republic (from 1996) and Belgium with limited time points (3). Therefore, data is insufficient to evaluate time-trend on the EU-wide scale. However, as described by Becker et al. (2013) no obvious trends of decreasing Cd concentrations have been observed in neither of the followed population groups in Germany. Similarly, also in Czech Republic, no significant trend was reported (Cerna et al., 2012).
4. Is there a link between high soil contamination with Cd and human exposure via dietary sources?	Within the work package WP5 (task 5.3) available data has been identified and applied into the mathematical models to describe the transfer from soil via fertilizers to plants (dietary source) and from plant to human via diet. Due to the scarcity of the external data available (soil, food, fertilizers, etc), the application was limited to the region-specific case study in Slovenia. The local case study is described in the Deliverable 5.5. The model enables to predict an oral intake via data on Cd concentrations in soil, phosphate fertilizers and food. Using HBM and food consumption data, the oral intake will be validated using the PBPK modelling (work in progress).
5. Which population groups are most at risk?	Dietary intake limit values are derived based on relationship between renal tubular impairments (proteinuria) and urinary Cd for women aged above 50 years (EFSA, JEFCA, ATSDR). Also, the HBM4EU HBM guidance value (HBM-GV) has been derived for the general population based on the increase in prevalence of elevated beta-2-microgloblin urinary levels as indicator of tubular proteinuria. The HBM-GV has been set at 1 ug/g crea, similar to the value of EFSA and the German HBM-I value. The kidney dysfunction is considered as the critical effect, but there is also evidence for low dose bone effects. The EFSA evaluation (2009) of the dietary Cd exposure showed that exposure of some subgroups, such as vegetarians, children and smokers and people living in highly contaminated areas could exceed the TWI of 2.5 ug/kg bw/week by about 2-fold. However, the revised assessment (EFSA 2012) indicated that the actual risk of adverse effects for an individual at current dietary exposure in the EU was low for adults, because the TWI was established based on an early indicator of changes in kidney function suggesting possible kidney damage later in life.

Policy Question	Short Summary of Results
	Within task 5.3 (Deliverable 5.5), evaluation of fractions of urinary samples exceeding the threshold value of 1 µg/g creatinine has been assessed for the available HBM data for women >50 years. The data indicated exceedance of the HBM guidance value for the higher percentile of exposure. Furthermore, attributable burden of disease related to Cd exposure was calculated in women aged > 50 years for chronic kidney disease, as a critical health effect, and osteoporosis at hip or spine. However, the estimations are preliminary and still premature for the use in policy recommendation. The main uncertainty arises from the questionable causality between Cd exposure and bone/kidney effects at low doses of exposure (below 5 µg Cd/g creatinine) that are commonly observed in the general European population. This has been outlined also in the Deliverables 13.4 and 13.5 elaborated within the task 13.2 with a purpose to establish exposure-health relationships. Variation in renal physiology is one of the main factors confounding the association at low exposure levels (co-excretion of low-molecular weight proteins and Cd). Moreover, normalization of Cd concentrations for diuresis is also a questionable issue, therefore the health risk assessment should rely on Cd measured in blood to compensate uncertainties related to Cd in urine (Stajnko et al., 2017).
6. Are the overall exposure levels (in different population groups) above any health-relevant assessment levels (HBM guidance values, TDI)?	We'll be able to answer this question once the complete data from the available datasets will be acquired (work within 10.4, described above). For the time being, we have concentration ranges available from the metadata of various studies, and from the literature. However, based on the EFSA evaluation of the dietary Cd exposure, mean exposure of adults across Europe is close to, or slightly exceeding the TWI of 2.5 ug/kg bw/week. The work conducted within task 5.3 (Deliverable 5.5) included evaluation of fractions of urinary samples exceeding the threshold value of 1 µg/g creatinine (critical Cd urinary level established by EFSA; HBM-I value and HBM4EU HBM guidance value) from the available HBM data (urinary Cd in women >50 years from Spain and France – BIOAMBIENT_ES and ENNS studies; and urinary Cd in women 35-45 years from 17 EU countries - DEMOCOPHES). The data indicated exceedance of the HBM guidance value for the higher percentile of exposure. These data, however, are not representative of the population at large and should be dealt with caution.
7. Has the regulation under REACH had the favourable impact like a reduction of GM/median concentrations?	Based on the very limited availability of the systematically repeated exposure data available (as explained under the time trends policy question activities), this question will be difficult to answer at this stage and will have to wait until repeated HBM exercises are performed in the future.
8. Are environmental quality standards for Cd in water sufficiently restrictive to protect human health from exposure to cadmium via the environment and via dietary sources?	Work in progress within WP10 and WP12 Following collection of HBM and dietary intake EU-wide data, and validation through the PBPK models, (drinking) water as a source of Cd will be included in exposure pathway to derive 'limit' value for Cd in water. In some of the countries (e.g. Slovenia) actual measurements in water and in population will allow direct links to be established.

Policy Question	Short Summary of Results
9. What is the maximum acceptable level for Cd in food stuffs?	Work in progress within WP10 and WP12 (similarly as above)
10. Can input be given to the decision as to whether the exemption for Cd in quantum dots under the RoHS Directive can be scrapped?	In general, population such a relationship is difficult to establish as currently the level of exposure is rather low and the time trends not established. Moreover, at this stage studies on occupational exposure in production line are also not available.

References:

Cerna et al. 25 years of HBM in the Czech Republic. International Journal of Hygiene and Environmental Health 220 (2017) 3–5.

Becker et al. German health-related environmental monitoring: Assessing time trends of the general population's exposure to heavy metals. International Journal of Hygiene and Environmental Health 216 (2013) 250– 254.

Stajnko, A., Falnoga, I., Tratnik, J.S., Mazej, D., Jagodic, M., Krsnik, M., Kobal, A.B., Prezelj, M., Kononenko, L., Horvat, M., 2017. Low cadmium exposure in males and lactating females–estimation of biomarkers. Environ. Res. 152, 109–119. doi:10.1016/j.envres.2016.09.025

9 Prioritised substance group: Diisocyanates

Policy Question	Short Summary of Results
What is current occupational exposure to diisocyanates?	Under WP8.5 a systematic review on the existing diisocyanate data was performed. This has been published as a paper by Scholten et al., Annals of Work Exposures and Health, 1-17, 2020. Although several studies were found describing exposures at different sectors, most of the studies were >10 years old and variable exposures were described. In addition, for example data on the use of diisocyanates in construction sector was limited. Study also highlighted the need for a harmonised approach to study and report biomonitoring levels. There is also a need to develop and test new, more specific biomarkers for the biomonitoring of diisocyanates.
What are the best markers to identify hazardous exposures to diisocyanates?	In addition to the systematic review by Scholten et al (2020), D9.5 (Prioritised list of biomarkers, matrices and analytical methods for the 2nd prioritisation round of substances) summarises the current state-of-art on the biomonitoring of diisocyanates. Although measurement of urinary diamines is currently “a golden standard”, this method is not specific for diisocyanates. However, it is possible to measure diisocyanate specific albumin or Hb adducts, which is recommended as a second option for biomonitoring. Also new methods for diisocyanate biomonitoring are under development as described in Scholten et al., 2020.
What is the likely impact of forthcoming REACH restriction/possible EU wide OEL of disocyanates?	This will be studied in the planned occupational diisocyanate study which will be performed in 2020-2021. The research plan for this second occupational study has been published as AD8.4 “Detailed research plan for the occupational diisocyanate and E-waste study” in the beginning of 2020. Laboratories performing analysis of different aniline compounds have been listed in D9.3 (Database of candidate laboratories for the 1st prioritisation round of substances) and ICI/EQUAS for aromatic amines started in June 2019 and the results are finalised in mid-2020. Laboratories passing the QA for urinary diamines will be candidates to perform U-diamine analyses in this occupational diisocyanate study.
What are the health risks and human health impacts of the current occupational diisocyanate exposures?	Health risk assessment of diisocyanates is under preparation in WP5.3. This will use available information on the dose-responses of diisocyanate induced asthma and existing information on diisocyanate exposure gathered from the literature and by WP10. PBPK model will be prepared under WP12 to link biomarker levels to external exposure levels. First version of the model will be finished in fall 2020 and the model will be tested and refined as part of WP8.5 occupational diisocyanate study. AOPs for diisocyanates are under development in WP13 to support human health risk assessment.

10 Prioritised substance group: Emerging substances

Policy Question	Short Summary of Results
Early warning of presence of hazardous chemicals in EU population?	Early warning methods for chemicals of emerging concern in HBM4EU implies new measurements in human samples of chemicals that have been recently introduced in the environment, but for which few Human Biomonitoring data exist (WP9, WP16), or detection of early warning signals of toxicity of chemicals present in the human population (WP14, WP16). HBM4EU develops and applies new methods for targeted analysis of these emerging compounds (WP9, AD9.1) to obtain robust quantitative information on the corresponding human internal exposure levels. HBM4EU complements this information with strategies and implementation of suspect screening (qualitative determination of an extended number of a priori known markers as a support to further prioritisation) and non- targeted screening (detection of unknown compounds as a support to new marker discovery) (WP16).In addition early warning of the presence of hazardous chemicals in the population is addressed through the use of effect biomarkers (WP14) that may signal biological imprints of chemical exposures. Specific “emerging” substances are being measured for the first time in biobanked urine or blood samples of the general European population that have been collected after 2014. The strategy is described in D8.4 (WP8). HBM4EU focuses on substitutes and alternatives for hazardous substances. As such capacity has been inventarised and expanded in Europe for targeted analysis of new phthalates (MCHP, DnPeP, DiDP, DiNP) with between 5 and 19 qualified laboratories depending on the metabolite. For the DINCH metabolites 7 and 8 laboratories were qualified depending on the metabolite. For BPF and BPS, respectively 13 and 18 qualified laboratories were identified and for organophosphate flame retardants (OPFRs) 4 to 5 laboratories were qualified depending of the metabolite. WP9 has selected the most suitable biomarkers for the first set of prioritised substances (D9.2) and for the second set of priority chemicals (D9.5). The phthalates and substitutes are currently being analysed in samples from 2950 children (NO,DK, HU,SK,SL,PL,EL,IT,NL,FR,DE) and 2900 teenagers (NO,SE,PO,CZ,SK,SL,EL,ES,FR,BE,DE). Organophosphate flame retardants (OPFRs) are being analysed in 2950 samples of children, the bisphenols are being analysed in 3165 adult samples. Some of the newer perfluorinated compounds are being analysed in samples from teenagers (NO,SE,PO,CZ,SK,SL,EL,ES,FR,BE,DE). Furthermore, new methods have been developed for detection of urinary metabolites of some reprotoxic classified and EU-regulated phthalates such as di(methoxyethyl) phthalate (DMEP), di-iso/npentyl phthalate (Di/nPeP), di-iso/n-hexyl phthalate (Di/nHexP) and di-C7-11 branched and linear alkyl ester phthalate (DHNUP). Smallscale ICIs are currently performed in order to expand the phthalate methodology for inclusion of these biomarkers for HBM investigations in the general population. Also, for analysis of CrVI in exhaled breath condensate (EBC) SOPs have been developed and a small sale ICI has been organised to allow implementation and generation of HBM data in the occupational studies. To identify new emerging substances, 5 European laboratories have joined forces and are applying their suspect screening capabilities to analyse 160 human urine/blood samples from various cohorts. They used a predefined list of suspect markers for which the expected detected signal characteristics have been inventoried within a MS reference laboratory. Based on this list, they identified dozens of markers from various substance groups including those for pesticides, plasticisers, UV-filters, and PFAS. Several were detected with high frequencies which demonstrates their widespread presence in the populations of various EU countries (D16.4).

Policy Question	Short Summary of Results
	Another proof-of-concept illustrating the WP16 outputs is concerning the particular suspect screening of pesticides and their metabolites in human urine samples. The aim is to annotate a maximal number of exposure markers present in urine samples that are being collected in mother-child pairs of 5 different countries and in 2 different seasons (D15.6). A user-friendly software module- haloseeker – has been developed as an open access resource to identify markers of exposure to halogenated substances in human samples. It allows to handle large and complex datasets which are generated by LC-HRMS (https://www.ncbi.nlm.nih.gov/pubmed/30758179). Special emphasis is on human samples collected in the early life period, considered as the most vulnerable period of life and an important time window that should be protected from exposure to hazardous compounds. A first success story was then obtained on breast milk samples where 4-hydroxy-chlorothalonil was identified without any a priori (D16.3). The same approach is applied to meconium samples. 25 Spanish placenta samples have been also analysed using untargeted LC-HRMS profiling, metal, steroid hormones and PFAS profiling and an array of bioassays has been applied on the samples (AD14.4). The results of the suspect and non-targeted chemical analysis will be combined with the outcome of the bioassays to link the exposure profiles with biological activity.
Inform REACH process to identify substances of potential concern?	The substances that fall under the 1st and 2nd priority substance groups of HBM4EU have been categorised according to existing knowledge of internal exposure (WP4). Category C substances are substances for which HBM data are scarce or doesn't exists. Category D substances are substances for which a toxicological concern exists but HBM data are not available. Suspect screening is recommended to explore the presence of these chemicals in human samples and further prioritise the necessary investment in term of developing quantitative methods and the inclusion of certain markers in HBM programs. Category E substances are substances not yet identified as of toxicological concern and for which no HBM data are available. Non-targeted screening approaches are needed to identify yet unknown substances. The results of this categorisation for individual compounds of the prioritised substance groups in HBM4EU can be consulted in the scoping documents on the substance specific web pages at the HBM4EU web site. It is the ambition of HBM4EU to move substances gradually towards category A as more information in being collected. To make progress for the identification of less well-known substances or yet unknown substances, a strategic EU database for QA/QC consolidated, harmonised and sustainable annotation capability for markers of exposure has been produced under HBM4EU. Lists of known emerging chemicals have been combined from 51 existing international databases of emerging chemicals to orientate the selection of compounds to be further characterised (D16.1). The list contains more than 70 000 MS-ready structures with unique structural and stereochemistry properties and their exact masses. Further 306,279 unique transformation structures are added to the list after simulation of phase 1 metabolism. The curated data serves as a reference for HRMS screening. The database can be used to putatively identify measured chemical features in untargeted HRMS, facilitating the large-scale detection of chemicals of emerging concern in human samples in Exposome research. To further identify chemicals of potential concern, QSAR predictions related to physicochemical properties, environmental fate, toxicity and Absorption, Distribution, Metabolism, Excretion (ADME) processes could be performed for 64,684 structures(AD16.5 and publication in press). This database complements existing metabolomics databases focused on markers of effect / endogenous compounds.

Policy Question	Short Summary of Results
Development of strategy for a non-toxic environment -> first step?	To identify new emerging substances in human samples, WP16 develops a data driven approach, a chemistry driven approach and a biology driven approach. Strategies for suspect and non- targeted screening are developed to identify yet unknown compounds of toxic concern in human biological matrices such as urine, blood, milk, meconium or placenta. A data driven approach: capacity on acquisition of high-resolution mass spectrometric data within the consortium is inventarised and brought together. The workflow for harmonisation and QAQC consolidation of the necessary reference MS data is laid down in AD16.4 “Annotation framework”. Reference mass spectrometric data are generated in a harmonised way so that they can be compared among laboratories and annotated to profiles that are generated when screening the samples. An inventory of screening techniques (AD16.1) and a first workflow for screening emerging chemicals (D16.2) has been published on the HBM4EU web site. A chemically driven approach: D16.2 highights crucial methodological questions of non-targeted analysis workflows including sample preparation, data acquisition, data mining and expert reviewing and proposes guidelines to implement NTA in Human Biomonitoring research. A set of QA/QC actions dedicated to sample collection, sample preparation and acquisition method specifically applied to the identification of chemicals of emerging concern in human matrices by non-target approaches is being developed and will be published. As a proof of concept non-targeted screening of halogenated emerging chemicals (incl. their metabolites) using gas/liquid chromatography coupled to high resolution mass spectrometry (GC/LC-HRMS) is developed and applied to various human matrices. A biology driven approach: combines suspect and non-targeted methodologies with effect directed analyses (EDA) (WP14). An overview of bioassays for analysing human samples and EDA approaches has been published (AD16.3) and a scientific publication is finalised. As proof of concept 25 placenta samples have been analysed with an array of bioassays including epigenetic markers (D14.4 and AD14.4). The results will be combined with the outcome of untargeted LC-HRMS profiling of the samples to link the exposure profiles with biological activity. In addition, effect markers are being selected and will be implemented in some of the HBM studies of WP8 as early warning signals for toxicity from exposure to multiple chemicals as occurs in real life. WP14 has defined effect markers as quantifiable changes in biochemical, physiologic or other parameters in the organism that occur as a result of exposure to chemicals. Criteria for selection of effect biomarkers (D14.1) and effect markers for the 1st set of priority chemicals (D14.2) have been identified. A distinction is made between novel effect markers, traditional effect markers with the novel markers relating more to early biological imprints of exposures, while the traditional markers are often clinical well validated markers that are reliable predictors of health risks but less specific for chemical exposures (D14.3).

11 Prioritised substance group: Flame retardants (FR)

Policy question	Results
1. What are current HBM levels of legacy/regulated FRs (e.g., PBDEs and HBCDD)? How do these compare to any historical records? Is the current legislative framework and proposed actions leading to a significant decline in restricted compounds and is this uniform across the EU?	In WP10, five legacy/regulated FRs were identified as the focus for statistical analysis. These are four polybrominated diphenyl ethers (BDE 47, 99, 153 and 209) and hexabromocyclododecane (HBCDD). Insufficient data was available through HBM4EU to evaluate spatial trends as planned, as well as insufficient data to evaluate temporal trends, although temporal trends have been evaluated and published for Norway (Thomsen et al. 2002; Thomsen et al. 2007) Sweden (Fängström et al. 2008; Norén et al. 2000; Meironyte et al., 1999; Darnerud et al. 2015; Lignell et al. 2015; Gyllenhammar et al. 2016) and Rome, Italy (Alivernini et al. 2011). These are summarised in the WP10 data analysis plan for flame retardants. Thus, the smaller set of data available through HBM4EU has been supplemented with literature data to allow a joint analysis of spatial and temporal trends in Europe and on a global scale, to place European population levels in a broader context. Literature data mining and statistical analysis is continuing in 2020, with the focus on maternal milk as a target biomonitoring matrix. A data analysis plan (task 10.4) on “Geographic Variations in Category A Flame Retardants in the European Population” reflects the planned analysis for these analyses. Further analysis on geographic differences in current-use FRs as well as determinants of exposure is planned for data generated by aligned studies. The combined interpretation of the legacy and current-use FR population levels will serve as a baseline to eventually evaluate whether declines due to chemical regulation are uniform across the EU.
2. What is the exposure of the European population to current use FRs? In particular, what is the exposure of sensitive sub-groups (e.g., infants and children)?	Under WP8, new aligned data is being generated for FR exposure in children age 6-11, for urinary biomarkers of organophosphate ester FRs, and serum biomarkers of BFRs by the end of 2020. Data will cover all four regions of Europe (North: Norway (300 urine and 300 serum), Denmark (300 serum); East: Slovakia (300 urine); South: Slovenia (150 serum), Greece (150 serum); West: France (300 urine), Germany (300 urine, 300 plasma), Netherlands (300 urine)). This will provide sufficient data to analyse exposure of the children to current flame retardants. Currently there is insufficient available data to evaluate this.
3 & 13. How do the levels of legacy FRs compare to levels of new/emerging FRs? Is any temporal or spatial trend observed? Can we relate this to use patterns and/or production volume? As FR market shifts towards replacement/alternative FRs, does human exposure reflect that trend? E.g., DBDPE as replacement for BDE209	Following the production of new aligned data under WP8, this analysis can be performed in WP10 in 2020. There is currently insufficient data to evaluate this question. This question is further being addressed through the pharmacokinetic (PK) modelling being conducted in WP12. As biomarkers of legacy FRs are typically quantified in serum/plasma, while many of the new/emerging FRs (i.e., organophosphate esters) biomarkers are quantified in urine, the measured levels cannot be directly compared. Therefore, PK modeling is crucial to enable comparison of the legacy and emerging FRs.

Policy question	Results
4 & 12. How does exposure to FRs differ between adults and children, males and females? What are the population groups most at risk?	There is not currently sufficient data to address this question, nor will sufficient data be produced under the harmonised WP8 framework, as the generation of new FR data will be limited to children ages 6-11. Therefore, this question is being addressed through integrated exposure modeling in WP12 (AD12.3). Exposure modeling for TCEP has identified highest estimated exposure to infants, however discrepancies between modeled exposure and measured urinary metabolites, particularly in infants and toddlers, indicate uncertainties and data gaps in FR exposure in infants and children. Toxicokinetics of TCEP exposure were assessed through INTERGRA PBTK model (AD12.5).
5. How does exposure differ by geographic area within Europe? Do countries/regions have different FR exposure levels?	This question is being addressed from WP10, initially for the restricted FRs for which there is sufficient data (4 PBDEs and HBCDD), and subsequently also for current use FRs when new data is generated through the aligned studies. The initial analysis will incorporate records of maternal milk for 10 European countries, and compare the geographic trends evaluated by the different matrices.
6. Are there one or more occupationally exposed sub-groups? What occupations are associated with high exposure to FRs?	The literature review completed within the framework of the scoping document (D4.2) has identified occupations with potentially elevated exposure to FRs (e.g., e-waste processers, computer repair, construction workers, some chemical industry workers, carpet installers). Occupational exposure to FRs has not been further addressed within the project. Occupational exposure will not be addressed in the aligned studies as the FR data will be only for children ages 6-11.
7 & 8. What are the relevant exposure pathways for FRs, e.g., diet, air, water, indoor environmental exposure? Is elevated exposure to FRs associated with particular consumption patterns or lifestyles?	Under the framework of WP12, a web-based exposure database has been developed to support the modelling of exposure towards better HBM data interpretation. Chemical-specific data include information related to the contamination levels in several environmental matrices such as ambient air, indoor air, water, soil, dust, as well food residues in various food items, and concentration in consumer products. In the current iteration of the exposure database this is available for brominated flame retardants, with geographically disaggregated data from Austria, Germany, Greece, Norway, Spain, Sweden and UK for environmental exposure, and Norway + general EU for dietary exposure, and general data for consumer products. This tool has been used in estimating population exposures, e.g, exposure through ingestion, inhalation and dermal contact was evaluated for TCEP (AD12.3, 12.5), highlighting non-dietary ingestion of dust as a major exposure pathway.
9. Do certain flame retardants co-occur in HBM matrices?	Yes, there is strong evidence based on existing HBM data (so far reviewed in literature during updating of scoping document, D4.2) that FRs occur in mixtures. A framework and statistical analysis plan has been developed within WP15 (AD 15.3) to provide a general concept and structure for how mixtures can be addressed. Mixture profiles for 26 flame retardants have been evaluated (D15.3) based on simulated data to gain insight into the determinants of mixture profiles in HBM data, but thus far is only simulated data and does not indicate direct biological relevance of these FR mixtures.

Policy question	Results
10. What current information is available regarding toxicity of FRs, both as individual compounds and as the mixtures of FRs typically occurring in indoor environments and diet?	Under WP13, FRs were classified and prioritised according to the availability of toxicological information and potential toxicity, as follows: 10 FRs with substantial toxicological information, 9 of which have toxicological concern (TCEP, TCIPP, TDCIPP, TPhP, TMPP, TBBPA, EHDPP, TNBP, TBOEP), 20 FRs without toxicological data in mammals, and 22 FRs with only scarce toxicological data. Molecular targets, health outcomes and potential AOPs identified for the 9 priority FRs with toxicological concern. The toxicity of FRs in mixtures has not yet been addressed.
11. Can exposure to FRs be linked with any adverse health effects?	This is the focus of WP13 and WP14. Under WP13, TBBPA was addressed as a first case, summarising existing toxicity information on TBBPA to link with adverse outcome pathways (AOPs) (D13.2) Evidence was found linking TBBPA exposure with thyroid hormone homeostasis, hepatotoxicity, carcinogenicity, neurotoxicity and teratogenicity. Similar exercises were completed for TDCIPP, linked with reproductive toxicity, and TPhP, linked with reproductive toxicity. Under WP14, a literature search was completed for biomarkers of effect for BFRs and OPEs. For BFRs 74 relevant publications were identified, covering 58 molecular/biochemical markers. Of those, 23 biomarkers were identified in at least 2 studies. For OPEs, the literature search identified 23 relevant publications. Ten biomarkers were proposed for implementation in Human Biomonitoring studies. Effect biomarkers were related to neurotoxicity, reproductive toxicity, and cardiovascular function. It was identified that there is a lack of information related to neurodevelopment.
14. What additional FRs should be prioritised for further information regarding exposure and/or toxicity? How can use and risk information be combined to identify and prioritise knowledge gaps for further assessment?	The scoping document (D4.2) highlighted 20 of 62 flame retardants with evidence of toxicity but insufficient HBM data. These are also candidate compounds to be prioritised for exposure assessment. These compounds are TPHP, TMPP, TCEP, TCIPP, TDCIPP, TNBP, TBBPA, and TBOEP (Cat. B), TEHP, EHDPP, DDC-DBF, ip-TPP, V6, 2,4,6-TBP and TDBPP (Cat. C and D) and DBNPG, TDBP-TAZTO, RBDPP, melamine polyphosphate and EBTEBPI are Cat. E. See D4.2 Section 5. WP5 built on the prioritisation of the scoping document by further classifying and investigating regulatory, risk evaluation and data availability for the 20 highlighted FRs. TCEP emerged as the most urgent FR to address. An HBM-based risk assessment was performed for TCEP aimed at the general population, using model-reconstructed external exposure starting with HBM data.
15. Can reference values be established for any FRs?	Based on the exclusion and partitioning criteria set by WP10, there is insufficient current data to establish reference values (limiting to general population, exclusion of hot spots, infants, children, adolescents and pregnant women/partitioning by matrix – serum or milk).

12 Prioritised substance group: Hexavalent Chromium (Cr VI)

Policy Question	Short Summary of Results
1. What is the current (last 5 years) exposure of the European population to Cr(VI)?	Inventory of studies holding Cr(VI) exposure data was obtained in WP7 with an online questionnaire which was distributed with the aim to identify existing HBM studies. Data available and data gaps are summarised in a report (see D7.1). Among all the priority substances Cr(VI) was one of the least studied substance; in particular, a total of 5 studies included Cr(VI) measurements: 2 of them from West European regions; 2 from South European regions and 1 in Israel. Although the preferred matrix for internal Cr assessment was blood, measurements were also available for blood erythrocytes, plasma, serum and urine spot random samples. A sampling frame to obtain EU recent HBM exposure data was developed by WP8 (see D8.1). In all the EU countries the lack of studies on environmental exposure to Cr(VI) was evident, due to the very low exposure levels of Cr(VI) in the general population. In AD8.1 an inventory of databases or datasets targeting occupational exposure to Cr in Europe (from WP 7.1 questionnaire) was reported. Six countries reported occupational biomonitoring data on Cr but the majority of data comes from the use of total Cr measurements. Since this is not specific for Cr(VI) it was decided to use new Cr(VI) specific biomarkers and to expand the scattered EU data on Cr(VI) (see below).
2. What is the level of exposure, environmentally and occupationally relevant to Cr(VI) in the EU population?	Cr(VI) has been identified as the first subject for a targeted occupational study under WP8 (see D8.5). Altogether 8 countries (Belgium, Finland, France, Italy, The Netherlands, Poland, Portugal, UK) volunteered to participate to the study on chromate exposure. Research plan for chromates study was published as AD8.2. After the publication of the research plan, Cr(VI) information sheet, information leaflets to the participating companies and to workers as well as informed consent forms for companies and workers have been prepared in collaboration with task 7.5. These were translated for local languages (French, Italian, Portuguese, Polish, German, Dutch and Finnish). In order to collect relevant background information on possible confounding exposures and operating conditions and risk management measures in place at the workplace, a questionnaire for data collection was prepared (Annex1, D8.5). In addition, to collect comparable data in a harmonised way, great efforts were made to develop Standard Operating Procedures (SOPs) for the collection, handling, sample storage and transfer for each of the biological and industrial hygiene samples covered within the Cr(VI) occupational study. SOPs for each specific matrix have been published in the HBM4EU on-line library. In the same time, an ICI/EQUAS for Cr analysis in different biological matrices has started within WP9 in order to select candidate labs for the analysis of samples of workers exposed to Cr(VI) and the list of candidates is available in D9.3. Results obtained in the chromate occupational study will allow to answer to the level of exposure to Cr(VI) in occupational settings. Analyses of samples of workers have been completed.
3. Does the exposure to Cr(VI) differ significantly between countries and population groups? What are the main reasons for differences in exposure?	In WP7 questions specific for Cr(VI) were identified to collect all the necessary information concerning countries (subdivision, GPS codes, town) and population characteristics (sociodemographic, dietary, occupational, lifestyle, environmental and health factors) with the aim to characterize possible differences among EU populations (see D7.3). In particular, exposure to metallic dust, type of work (surface treatment, handling metals, etc.), body modifications (piercings, tattoos), metallic jewellery on the skin, type of food and drink consumed before the sampling, have been identified as the main possible reasons for differences in Cr(VI) exposure.

Policy Question	Short Summary of Results
	In addition, WP10 has developed a substance-specific statistical analysis plan for Cr(VI) (see D10.2, D10.5 and D10.6), including the definition and harmonisation of the variables, the statistical test to be applied, the specific procedure for calculating EU reference values, uncertainty analyses, data descriptions, and visualisations. Variables on general exposure levels, geographic comparisons and exposure determinants were defined in relation to Cr(VI) exposure (like SES, education, type of area of residence, density of traffic in the residential area, smoking, passive smoking, cotinine, local food, seafood, tattoo, jewellery, nutrients). These variables were mandatory in the statistical analyses to address Cr(VI)-specific differences among countries and population groups. Despite these protocols and procedures, the poor availability of HBM data on Cr(VI) in different countries and population groups does not allow to answer to this policy question so far. Within WP8, observations from occupational exposed cohorts could bring further evidence.
4. Is there a significant time trend of Cr(VI) levels in existing population studies?	A protocol for examination of the temporal trends of Cr has been elaborated (WP10; D10.2). However, no study was identified that have repeated Cr measurements available. Therefore, data are insufficient to evaluate time-trends on the EU-wide scale and to answer to this policy question.
5. What are the groups at risk?	The literature review within the framework of the scoping document (D4.2) and of deliverable AD8.1 has identified occupations with potentially elevated exposure to Cr(VI). In EU the estimated number of Cr(VI)-exposed workers in 2012 was ~786,000, with the largest numbers exposed to welding. Other major uses of Cr(VI) include metal plating, manufacture of pigments and dyes, corrosion inhibitors, chemical synthesis, refractory production, leather tanning, and wood preservation. Within the WP8, results of chromate study will aid to evaluate the Cr(VI) exposure in some of the most exposed classes of workers (chromium plating and welding). The main uncertainty for the evaluation of risk arises from the lack of knowledge on the relationship between Cr(VI) exposure and health effects. This issue has been reported in WP13 with a purpose to establish exposure-health relationships. WP13 give a detailed overview of the available knowledge on AOPs for Cr(VI) (D13.4 and D13.5) and subsequently summarised key knowledge gaps emerging from previous research. Specific activities, studies or other relevant steps to fill the missing information on Cr(VI) have been proposed in D13.5. In D5.5, it has been exemplify the inclusion of HBM data in risk assessment (RA) and health impact assessment (HIA) strategies. RA was performed also for Cr(VI). Since the data were limited to Finland and pre-authorization period, this policy question can be better answered when new, EU wide data on occupational exposure to Cr that will appear from the chromate study under WP8.
6. Are the overall exposure levels (in different population groups) above any health-relevant assessment levels (HBM guidance values, TDI)?	Relevant HBM guidance values for the exposure to Cr have been reported on a national basis, but not at EU level. In the scoping document (D4.2) all the available limits have been reviewed. In Span, a biological limit value (BLV) for total Cr concentration of 10 µg/L in urine measured during a shift and 25 µg/L at the end of the workweek has been reported (INSHT, 2016). In the UK, a biological monitoring guidance value (BMGV) of 10 µmol/mol creatinine in post shift urine was established (HSE, 2011). In Germany, DFG established the DFG-EKA values (biological exposure equivalents for carcinogenic substances) for Cr(VI) that set the range of total Cr in urine (form 10 µg/L to 40 µg/L) and in erythrocye fraction of whole-blood (form 9 µg/L to 35 µg/L) if soluble alkaline chromate of a certain

Policy Question	Short Summary of Results
	concentration and/or hexavalent welding fumes (only for urine) were inhaled over a work shift (DFG, 2015). Management of Cr(VI) formed during the welding process is achieved by compliance with occupational exposure limit values (OELs). The recent binding OEL set under EU Directive 2004/37/EC on the protection of workers from the risks related to exposure to carcinogens or mutagens at work is 0.010 mg/m3 for a period of 5 years after the date of transposition of the directive; after that period a limit of 0.005 mg/m3 will apply. For welding or plasma-cutting processes or similar work processes that generate fumes, there is a derogation, with an OEL value of 0.025 mg/m3 until 5 years after the transposition date and after that period the limit will be 0.005 mg/m3. On the other hand, in France and the Netherlands, an OEL of 1 µg/m3 has been set for Cr(VI) in all uses. These are the most stringent OELs currently set in workplace in EU. This policy question can be better answered when data on occupational exposure to Cr(VI) will be available under WP8.
7. Has the regulation under REACH had the favourable impact like a reduction of GM/median concentrations?	Cr(VI) is one of the most important occupational carcinogens, which has been shown to cause lung cancer in humans. It is currently an issue in the EU since some Cr(VI) compounds are authorised under Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). The current occupational biomonitoring data on Cr(VI) is scattered and its coverage is limited. Moreover, based on the very limited availability of the systematically repeated Cr(VI) exposure data available (as evidenced under the time trends policy question activities) this question cannot be answered at this stage. In future, the data from the chromate study would support not only implementation of occupational health and safety legislation but also EU chemicals legislation (REACH).
8. What are the current HBM methods for Cr(VI)?	Within the WP9, an inventory of available methods and matrices suitable for Cr measurements have been reported (see D9.2). This inventory, covering articles published in the years 2010-2017, revealed the presence of 16 references in total, but only 8 fulfilled the analytical requirements. Chromium is analysed in urine, whole blood, exhaled breath condensate (EBC) and red blood cells (RBC). All described methods use ICP-MS, GF-AAS, EAAS and AAS, and the most frequent sample preparations are: liquid extraction, centrifugation and clean up using strong acid. In conclusion, the preferred technique for Cr determination is ICP-MS. An alternative is the speciation of Cr (VI) and Cr (III) by coupling ICP-MS to liquid chromatography. Within WP11, information about biological matrix previously used for the Cr(VI) measurement use in previuos studies and obstacle to link HBM data and health was given (D 11.1). Within WP8 and WP9 harmonised methodology for total Cr and Cr(VI) analyses including collection, conservation, transport, preparation and analysis of biological (urine, blood and exhaled breath condensate EBC) and industrial hygiene samples (air and wipes) were developed (as above reported). Moreover, SOPs were developed for any of these matrices (as above reported). In the same time, within the WP9, laboratories performing laboratory Cr analysis have been tested through QA/QC schemes for the determination of Cr in urine, whole blood and serum. The 3° round of proficiency tests has been completed. Additionally, a few laboratories have set up the methodology for the analysis of Cr(VI) in EBC (D9.7). Moreover, for EBC-Cr(VI) a small-scale interlaboratory comparison to ensure the quality of the analysis has also been conducted (D8.5). The first list of approved laboratories for Cr analyses is available (see D9.3) and all qualified laboratories were asked for information on price, capacity, time frames and technical details of Cr analyses (AD9.3).

Policy Question	Short Summary of Results
9. Which are the appropriate biomarkers for Cr(VI)?	Regarding biomarkers of exposure, scoping document (D4.2) and deliverables (AD8.1) identified the urinary Cr levels as a measure of total Cr exposure as Cr(VI) is reduced within the body to Cr(III). The analysis of plasma is indicative of total Cr exposure because Cr(VI) may be reduced to Cr(III) in the plasma. Cr measurements in red blood cells (RBCs) were selected as the most suitable biomarker for the analysis of Cr(VI) exposure because Cr(VI) passes through cell membranes, while Cr(III) does not. The analysis of the exhaled breath condensate (EBC) was selected as a very good biomarker of occupational exposure to Cr(VI). Currently the most appropriate matrix for the determination of Cr(VI) is the analysis of RBC because only Cr (VI) can enter into them. An alternative to invasive matrices is the determination of Cr (VI) and Cr (III) in EBC to measure exposure to Cr(VI) compounds long after exposure. Furthermore, Cr-RBC correlated with Cr(VI) in exhaled breath condensate (EBC). Concerning biomarkers of effects, in WP8 (task 8.5) the chromate study includes also the collection and analysis of samples for several effect biomarkers analyses. Effect markers planned to be analysed in chromate study (see D8.5) were reticulocyte micronuclei (MN), MN in peripheral blood lymphocyte (in collaboration with WP14), comet assay in leukocytes, global methylation analysis (and specific epigenetic markers), telomer length in blood, metabolomics studies (urine), oxidative stress biomarkers in urine. Work is in progress. Moreover, a literature survey was performed for Cr(VI) in order to create an inventory of available biomarkers of effects for this specific exposure. The results of that survey are presented in AD14.5. The traditional effect biomarkers included oxidative stress (e.g., malondialdehyde) and genotoxicity (e.g., micronucleus analysis) markers. Among the novel effect biomarkers, those relying on gene expression and epigenetic effects (e.g., DNA methylation analysis) were identified as the most informative ones.

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13 Prioritised substance group: Lead

Policy Question	Short Summary of Results
1. What is the concentration of lead in the human blood nowadays (after phasing out leaded petrol) in the countries of Europe?	The work is progress in WP10. The first draft protocol has been elaborated for the statistical analysis. The relevant data and publications were collected.
2. Do blood lead levels of both adults and children still indicate permanent existence of lead exposure?	The work is progress in WP10. The first draft protocol has been elaborated for the statistical analysis. The relevant data and publications were collected.
3. What are the sources of still existing lead exposure in different countries of Europe?	The work is progress in WP10. The first draft protocol has been elaborated for the statistical analysis. The relevant data and publications were collected.
4. What kind of exposure sources are the most important for the children of various age groups and the younger or older adult population?	The work is progress in WP10. The first draft protocol has been elaborated for the statistical analysis. The relevant data and publications were collected.
5. Taking the hazard from transplacental lead exposure of the unborn child into consideration, what are the blood lead levels of pregnant women?	The work is progress in WP10. The first draft protocol has been elaborated for the statistical analysis. The relevant data and publications were collected. Unfortunately, there is only a few relevant studies.
6. Taking the presumably low concentration of lead in blood, is it feasible to measure blood lead levels in children from as small amount of blood as it can be gained from capillary samples? What criteria should be applied in order to avoid contamination from outside sources?	As lead is not investigated in the HBM4EU studies this question could not yet be addressed.
7. As it is difficult to connect later outcomes with exposures, which biomarkers of effects can be used in relation to effects caused by lead exposure?	A review on the biomarkers of effect associated with lead exposure and neurodevelopment is in progress. The literature has already been checked and 24 eligible studies were identified. The investigation of other health effects might be interesting; however, neurodevelopment is the major health effect related to exposure to lead.

14 Prioritised substance group: Mercury and Methylmercury

Policy question (PQ)	Short Summary of Results
1. How effective are policy actions to reduce human exposure to mercury in Europe? (Including the EU's Strategy on Mercury and the Minamata Convention, which was ratified by the EU and Member States)?	WP4: The "Mercury & Methylmercury" Chemical Group was prioritised for action within HBM4EU in the 2nd round of prioritisation, was approved in May 2018 and a Chemical Group Leader was appointed ("D4.5 - Second list of HBM4EU priority substances and Chemical Substance Group Leaders for 2019-2021"). A scoping document was developed, which summarises the state of the art, identifies knowledge gaps, presents policy questions and suggests actions to be carried out in the frame of HBM4EU to address them ("D4.6 - Scoping documents for the second-round priority substances"). The scoping document is updated yearly ("D4.7 - Scoping documents for 2020 - 2nd set of priority substances - Update of policy questions and related HBM4EU activities"). Work Package 2 (WP2): To explore how HBM4EU might contribute at global level, the WPL2 and the CGL-Hg attended the 2nd Conference of the Parties (COP2) to the "UN Minamata Convention on Mercury", which took place in November 2018, in Geneva. The CGL presented HBM4EU and its work on mercury in the frame of a Knowledge Lab on "Mercury in the European Environment and Population", which was organised by the EEA. Ways in which HBM may contribute to the effectiveness evaluation of the convention and possible collaborations with the World Health Organization to support global harmonisation of mercury biomonitoring were explored. The HBM4EU work on mercury was presented at international conferences (e.g. 14th International Conference on Mercury as a Global Pollutant, CLIMATICO 2019). The 4th HBM4EU newsletter (of April 2019) featured mercury as a prioritised Substance Group in HBM4EU. In 2021, WP2 will prepare a policy brief on the mercury priority group, which will summarise toxicity, exposure, and policy status, any updates in legislation and relevant HBM4EU research results. The overarching activities in Work Packages (WP) 2,4,5,6,7,9,10,11,12,13,14 support the effectiveness evaluation of current policies by establishing a baseline exposure of European populations and tools/expertise to harmonise exposure assessment in Europe so that time-trends can be followed, evaluated and interpreted regarding health risks. More details are given elsewhere in this table. An overarching meeting on mercury was held in October 2019 in Berlin. It fostered discussions and provided the forum to discuss the ongoing and planned activities under the HBM4EU. Representatives of the WPs presented current progress and future work was coordinated. At the overarching meeting, a possible new aligned study on mercury was discussed. This was in continuation of a survey of the National Hubs, aiming to assess the interest of the countries in a new aligned study on mercury and to define its objectives. A total of 16 countries expressed potential interest. The outcome of these discussions, was a decision to propose to the HBM4EU Management Board the implementation of an aligned intervention study in 5 high-fish consuming countries (IS,ES,PT,EL,CY) with the aim to control fetal exposure to mercury by combining HBM with the provision of suitable dietary to pregnant women. The CGL, representatives of the five countries and of relevant HBM4EU WPs have been in discussions with the Management Board to elaborate a study plan, which may be pursued. If implemented, this study will provide knowledge relevant to policy questions 2,4,5.

Policy question (PQ)	Short Summary of Results
2. How can harmonised, validated and comparable information be collected and transferred to support and evaluate current policies?	Work Packages 7, 9 and 10 developed a frame to assure the quality of new collections of biomonitoring data and of existing samples and data, which are made available to the project. Work Package 7 (WP7) developed strategies, Standard Operating Procedures (SOPs) and materials, which can support the harmonised recruitment, sampling (including sample exchange), questionnaire implementation and communication with participants in HBM studies, taking into account ethical and personal data protection requirements. Relevant deliverables include: “AD7.1: Upgrade of the strategy for human sample exchange, including SOPs”; “D7.2: Strategy and SOPs for human sample exchange, including ethical demands”; “D7.3: 1st prioritisation Report on survey design: Study protocols, SOPs and Guidelines, tailored and transferred questionnaires for recruitment and sampling”; “D7.4: 1st material for communication to participants, including informed consent”; “D7.7: 2nd set of materials for communication to participants, including informed consent”; "AD7.2: Literature research and concept for a sample quality study on impact of thawing and freezing on integrity of human samples for selected 1st and 2nd priority substances”; “D7.6 - 2nd prioritisation Report on survey design: Study protocols, SOPs and Guidelines, tailored and transferred questionnaires for recruitment and sampling”. Work Package 9 (WP9) elaborated criteria for the identification and selection of the best exposure biomarkers and matrices and for the evaluation of analytical methods, which can be used in European HBM surveys (see “D9.1: Criteria for prioritisation of biomarkers, matrices and analytical methods”). Based on these criteria, a prioritised list of biomarkers, matrices and methods for the assessment of human exposure to mercury and methylmercury was elaborated (see “D9.5: Prioritised list of biomarkers, matrices and analytical methods for the 2nd prioritisation round of substances). The matrices of choice for both mercury and methylmercury were identified to be urine, whole blood and hair (with the latter being an easily available, non-invasive and inexpensive matrix for the estimation of methylmercury exposure). Dried blood spot samples and meconium are new matrices, which may prove valuable for the evaluation of newborns exposures based on recent literature reports. The recommended analytical techniques for the determination of methylmercury are AAS (atomic absorption after generation of cold vapor of Hg) and ICP-MS. A list of 43 candidate laboratories for the determination of mercury was elaborated in “D9.6: Database of candidate laboratories for the 2nd prioritisation round of substances”. Work Package 10 (WP10) elaborated a Data Management Plan (DMP) in D10.1, which describes the data management life cycle for all datasets to be collected, processed and/or generated by HBM4EU (including criteria/methods and standards for data collection, data handling and sharing, data storage, data accessibility, while respecting ethics/legal requirements). This Plan includes the HBM4EU Data Policy (Attachment to the DMP D10.1). WP10 also developed a Statistical Analysis Plan (D10.5), which includes issues common to all HBM analyses, description of time-trends, geographical comparisons, exposure determinants, exposure distributions and reference values, uncertainty analysis as well as a specific statistical analysis plan for the mercury priority substance group. All materials, which have been developed to support harmonised practices, are publicly available to the whole consortium and any other interested party, via the online library, which is managed by Work Package 2 (WP2).

Policy question (PQ)	Short Summary of Results
3. What biomonitoring and exposure data on mercury (and its species), relevant to the European population, are currently available and what new data are needed to address policy-related questions?	The Scoping Document on Mercury and Methylmercury, which was developed in the frame of Work Package 4 (WP4), presents currently available data, and identifies open policy questions and data gaps to fill in order to address them. The policy questions and proposed actions took into consideration feedback received from the internal experts, the EU Policy Board and the Work Package Leaders. As a result of discussions of the knowledge gaps and policy needs with the NHCPs and relevant consortium partners, a proposition was elaborated for an aligned intervention study in 5 high fish-consuming countries, targeting pregnant women (see under PQ1). Work Package 7 (WP7) reported (in D7.5) European ongoing activities, existing data and data gaps for the 2nd prioritised substances, including mercury. This information was collected through a questionnaire distributed via the National Hub Contact Points of participating countries in HBM4EU and completed by Principal Investigators. A total of 25 European studies concluded within the last 10 years, ongoing, or planned for starting up in the next five years included assessment of mercury. Work Package 10 (WP10) achieved progress regarding the inclusion of existing European HBM data in the European commission's Information Platform for Chemical Monitoring (IPChem). Deliverable "D10.6 - 2nd annual list of exposure distributions and/or European reference values" (published in 3/2020) presents the statistical analysis of aggregated existing HBM data on mercury, which were made available to the HBM4EU consortium by 2019. For total mercury, the following available studies were analysed: Using urine: Nine studies on adults (>20y; East Europe: 3, South: 1 West: 4) and 2 studies on children (3-11y; East Europe: 2).Using Blood: Nine available studies on adults (>20y; East:4, South:2, West:2, North:1), two studies on children (3-11y, East:2) and one study on toddlers (1-2y) (East:1). Breast Milk: One available study from South Europe and one from East Europe. Hair: Twelve available studies on adults (>20y; East:4, South:4, West:3, North:1), nine studies on children (3-11y, East:4, South:2, West:2, North:1) and three studies from West Europe on teenagers (12-19y). Fewer studies were available with data on methylmercury (MeHg): Using Blood: One study from East Europe on toddlers (1-2y); Using Hair: Two studies on adults (>20y: East:1, West: 1) and three on teenagers (12-19y, West:3). Where possible, data were stratified by sex and educational level. For all available studies, the P05,10,90,95 and the LOQ and Highest values were described. In summary, the exposure to mercury is higher among participants of high educational status as compared to low educational status. The difference isn't pronounced for participants with middle educational status. As more data become available to the HBM4EU consortium, this work will be updated.

Policy question (PQ)	Short Summary of Results
4. What is the geographic spread of the current exposure and how does it relate to different exposure sources (environmental; contaminated sites; dental amalgams; dietary, including different species of sea-food)? Ideally, this should capture the exposure of highly exposed populations (e.g. high seafood consumers with distinction of populations consuming predator fish from those with low/no consumption of such fish, such as Southern & Northern Europeans, European arctic populations), but also of low-exposure populations for comparison. **Which populations remain vulnerable to health impacts from mercury exposure and how can they be protected?**	The scoping document on mercury, developed under Work Package 4 (WP4), summarises a lot of available data related to the geographic spread of human exposure, reviews determining factors, provides some information on available relevant cohorts and identifies data gaps. The exposure is higher in coastal populations and correlates with high fish / seafood consumption. The formulation and provision of suitable dietary advice to vulnerable populations should be taking into consideration the contamination levels in different species of fish, as well as the documented nutritional benefits of fish consumption. Work Package 10 (WP10) collects, integrates and makes available existing HBM data on mercury into IPCHEM. The existing and available HBM data are analysed to assess the baseline exposure of Europeans to total and methyl mercury and the determinants of the exposure. In “D10.6 - 2nd annual list of exposure distributions and/or European reference values”, the available mercury data from European studies, provided to the project by the participating countries, were treated statistically in a harmonised way. Distribution plots were prepared, presenting the exposure data according to sex, European region, age group and educational level (as a measure of socioeconomic status). See also under PQ3. Work Package 12 (WP12) prepared an integrated exposure modelling platform, which provides a web-based computational environment that brings together the different tools available from the HBM4EU consortium to address all the aspects of the full chain for aggregated exposure assessment in environmental and occupational settings (see “AD12.4 Conceptual design of the integrated computational platform”). The vertical modules include (a) the model run settings module, which serves to define some general settings for each platform run and the overall configuration of a specific platform simulation, (b) the multimedia model module, for the estimation of environmental media concentrations in different environmental matrixes, (c) the microenvironment module, for estimating chemical environmental concentration for indoor locations, (d) the exposure scenario definition module, which enables the development of an exposure scenario for the population group(s) selected, including all possible exposure routes and (e) the internal dosimetry module, which aims at estimating the internal doses of a chemical and its metabolites. The whole concept will be based on a suite of three different PBTK models according with their level of detail and complexity (the simpler PK model for data poor chemicals, the generic PBTK model and the life-stage changing mother-foetus generic PBTK model). The integrated exposure modelling platform will be optimised by updating the exposure model parameterisation for mercury using available data and will be used to identify the internal exposure to total mercury and methylmercury for various population groups.

Policy question (PQ)	Short Summary of Results
5. How can the public be informed and how can public awareness and education be raised regarding the effects of mercury on health and the environment and about management options? **What advice should be given regarding dietary recommendations to vulnerable Europeans (e.g. pregnant women, infants, high sea-food consumers) and other stakeholders (e.g. health practitioners, policy makers) to reduce exposure to mercury while in keeping with nutritional requirements and cultural dietary preferences? Ideally, this should consider the different types of foodstuff (e.g. types of seafood) consumed in different parts of the EU, the toxicity and occurrence of the different mercury species in different foodstuff and the positive effects of n-3 long-chain polyunsaturated fatty acids in fish and of micro nutrients (e.g. selenium) in the diet. Related to this, how can HBM4EU results support policy decisions at EFSA and ECHA?**	Work Package 2 (WP2) created a dedicated webpage on the Mercury Priority Substance Group on the HBM4EU website. It includes the scoping document and provides the venue for disseminating a summary of HBM4EU results on the mercury group. Different communication products are being prepared and dissemination actions are elaborated as the work on mercury progresses in the project. A factsheet on mercury for lay audiences was drafted and mercury was featured in the 4th HBM4EU Newsletter (April 2019). In September 2019, a Facebook Live Session on HBM4EU was broadcasted, during which the project coordinator (UBA) and an EEA expert discussed the project. The event engaged citizens from all over Europe, many of whom expressed concerns about mercury and asked related questions. The HBM4EU work on mercury was presented at international scientific conferences and at the 2nd Conference of the Parties to the UN Minamata Convention on Mercury. All open-access deliverables referenced in this summary of results on mercury are accessible on the HBM4EU online library (https://www.hbm4eu.eu/deliverables/). Videos for lay audiences were prepared and are available on the HBM4EU website, to inform citizens about the problem of chemical exposures and how HBM4EU works to inform European policies for safer chemicals management. Work Package 4 (WP4) organised Citizen Focus Groups in Austria, Portugal, Ireland and the United Kingdom with the aim to gain information on the interests, needs, and questions of European citizens regarding exposure to chemicals in their daily lives and their opinions about possible future actions on Human Biomonitoring. Mercury was among the top five environmental pollutants causing concern to the participating citizens, who regard it as a priority which should be addressed in Human Biomonitoring studies (see “AD4.2 Report of the citizen’s focus groups”). An Online (questionnaire) Survey was also organised in the countries, which carried out the focus groups, with the aim to understand what EU citizens know about Human Biomonitoring in general, as well as their needs and concerns. Four more countries will carry out similar citizen consultations in 2020 (The Netherlands, Hungary, Denmark and Cyprus). Work Package 11 (WP11) reviewed known relations between substance (including mercury) exposures and health outcomes, based on the information from the scoping documents of WP4. Scientific review-manuscripts are in preparation for Metabolic syndrome, diabetes, osteoporosis and asthma, with the aim to raise awareness in health professionals regarding the health effects of environmental contaminants. Also, WP11 evaluated opportunities and obstacles related to linking HBM, health surveys and administrative data sources (Deliverable D11.3), generated an inventory of European health studies, which could be linked to HBM studies (D11.1) and provided SOPs/guidelines for combining HBM/health studies and for obtaining information regarding identified health effected related to mercury exposure. A study plan was elaborated, for a new aligned study in five fish-consuming European countries, with the aim to develop and provide suitable dietary advice to pregnant women and to evaluate the effectiveness of the intervention using Human Biomonitoring (see under PQ1). Also see below under PQ6, for relevant work by Work Package 5 (WP5).

Policy question (PQ)	Short Summary of Results
6. At what level of exposure to different mercury species and to total mercury are health effects likely to occur? Current guidance values were based studies of the Faroese people, who have a diet that is unique and does not relate to food consumption patterns in the EU. This important issue has not been given proper attention to date.	Work Package 5 (WP5): To facilitate the interpretation of HBM data in terms of health risks, WP5 developed and refined a procedure for the derivation of HBM-Guidance values (HBM-GV) for the general population and for workers (submitted for publication). Work is planned in 2021 for the derivation of HBM-GVgen.population for mercury, provided that sufficient epidemiological / toxicological / toxicokinetic data are available. If not, recommendations will be elaborated for data needed to fill the gaps. In the frame of task 5.3, a draft methodology was developed and will be followed in 2020-21 for an improved risk assessment for vulnerable populations. It will identify available and suitable European data, including published dose/response data, will evaluate the weight of different exposure sources in Europe, and the most suitable matrices and methods to measure exposure to infer associations between different matrices in different exposure situations. Work Package 11 (WP11) develops guidelines to support the standardisation of measurements and comparability of collected health data relevant to mercury in future studies. A literature search by Work Package 12 (WP12), presented in “AD12.8 Review of available models for the 2nd set of prioritised substances”, found that methylmercury and excretion of organic and inorganic mercury have been studied in vivo in humans and animals and that toxicokinetic (PBPK and conceptual) models for methymercury have been developed in humans to relate external exposure to parent or derivative compound concentrations. Exposure to mercury is studied indirectly by exposure assessment of methylmercury through inhalation and/or oral routes and emphasis is given on the early stage of development during pregnancy, fetal development and lactation. All the studies presented modified PBPK models based on specific literature and can be used, if modified, to study other sensitive population groups. The cited research focuses mainly exposure to methylmercury. In AD12.10 (to be published in 2020), available experimental and in silico data were reviewed to parameterise properly TK and PBTK models for 2nd priority substances, values of key toxicokinetic parameter were identified and differences in toxicokinetics that depend on gender/developmental stage were reported.

Policy question (PQ)	Short Summary of Results
7. How does exposure relate to the manifestation of adverse health effects? - What are possible health effects resulting from chronic low exposure to mercury and its organic compounds (such as from food consumption and dental amalgams)? This type of exposure is the most relevant for Europeans and can be addressed by speciation analysis of biobanked samples from existing cohorts and associations with adverse health effects. **- What factors make people more susceptible to the development of health effects due to mercury exposure?**	Work Package 13 (WP13) carries out work to establish causal links between exposures and health. Tasks 13.1 and 13.2 jointly developed a WP13 Strategy on addressing the policy questions related to the 2nd list of priority compounds (see "AD13.1 Strategy on how to address policy questions related to the 2nd list of priority compounds"). A mercury-focus group was established and a leader for the substance group within WP13 was identified and actions on how to address the mercury policy questions were planned. In AD13.6 "Answers to exposure-health policy questions for the 2nd priority compounds", the ongoing work is summarised: Existing cohorts are used to assess Hg impact on neurobehavior taking into account co-exposures to other elements with neurotoxic potency (Pb,As,Mn) and OP pesticides and also beneficial elements (including Se, Zn) and to investigate the allele frequencies of related SNPs across Europe and how this may contribute to contradicting associations between exposure and health outcomes found so far. This will be done in association with WP10. A literature search is also underway, to evaluate new evidence from the epidemiological literature since EFSA's 2012 opinion, in order to evaluate if new developments have occurred to address the identified data gaps and if current knowledge supports or contradicts the EFSA's conclusions from eight years ago. The output of this work will be presented in a peer-review publication. Work Package 14 (WP14) carries out work to identify the most suitable biomarkers of effect for mercury through a focused literature search of mercury-related human and animal studies and reported health endpoints (see D14.5 - Selection criteria and inventory of effect biomarkers for the 2nd set of substances). Comprehensive literature searches with defined search terms for mercury/methylmercury and selected health endpoints (reproduction, neurodevelopment, metabolic & cardiovascular, immune/allergy, endocrine, and cancer) were contacted in the PubMed/MEDLINE database (D14.5). To focus the literature search on novel biomarkers and to reduce the number of articles, search terms related to OMICS, epigenetics or biomarker-related were included, while exclusion terms were used to eliminate articles with irrelevant focus. The most relevant effect biomarkers used in epidemiological settings are summarised, prioritised and linked to the available experimental or AOP support (D14.6). BDNF, oxidative stress and many epigenetic and gene expression markers were found to be correlated with higher exposure to mercury in human studies. Given the wide amount of specifically deregulated genes and epigenetic effects identified, future efforts should prioritise the most interesting molecular targets based on mechanistic and AOP information. The output of this work will be reported in a review article, which is under preparation.

15 Prioritised substance group: Mixtures

Policy Question	Short Summary of Results
1. What is the information need of regulatory bodies and stakeholders?	The information needs on mixtures of regulatory bodies and to a lesser degree of other stakeholders was explored through a combined approach of literature review (including policy documents), development of a semi-structured interview protocol, interviews of experts and policy makers and interpretation of the results. The effort also benefited from HBM4EU's participation in the Horizon2020 Joint Mixture Project Cooperation, particularly from the joint meetings where experts and policy makers discussed current issues in research and management. The outcomes of this work are described in detail in D15.1. Focus in this deliverable was on policy makers. Basically, the approach was as follows. For the governance of mixture risks, we formulated a number of 'rational statements' and questions that are relevant to HBM4EU. Subsequently, a set of questions were derived from the statements; these were used for semi-structured interviews with international experts. Second point of departure was to consider mixture risks as a 'systemic risk' problem (this also emerged in the discussion of the Horizon2020 Joint Mixture Projects). Systemic risks in the context of environmental health are complex risks to health embedded in wider environmental, social, economic and political systems (for references see D15.1). Systemic risks require more integrated and possibly precautionary approaches to risk governance. One of the characteristics of systemic risk problems is that systemic risks are under a distributed responsibility: everyone is responsible for a part of the system but no one has the legitimacy to act on the entire system; this is clearly the case for the regulation of mixtures across different regulatory silos. Other characteristics of systemic risks are the inherent substantial uncertainties, complexity and ambiguity of the problems. "Complexity" should here be understood as the difficulty to identify and quantify causal relationships between a variety of potential hazards and the multitude of potential effects following exposure. "Uncertainty" pertains to a situation where the type or nature of any adverse effects, or the likelihood of these effects, cannot be described precisely. "Ambiguity" refers to a situation where several legitimate and meaningful interpretations of accepted risk assessment results coexist. Subsequently, it is quite common to encounter ambiguity about normative values and ethical norms. The conclusions from the literature and interviews were: • Mixture can be viewed as 'systemic risks' given the properties of uncertainty, complexity, and ambiguity and the general 'embeddedness' of chemicals in daily life; risk governance approaches for mixtures should therefore be targeted as such and the contextual aspects may require tailored approaches instead of generic regulation. • The information needs from policy makers and experts is still rather diffuse and unarticulated. • As can be expected from the literature on systemic risks, views on responsibilities and criteria to guide risk reduction strategies vary considerably; this warrant further exploration of views and mental models held by the stakeholders involved. • A broader dialogue on information needs for mixture risk governance with stakeholders is needed, but as yet not planned. Any such exercise, within HBM4EU, should be done in conjunction with Pillar 1.

Policy Question	Short Summary of Results
	On the basis of the literature and interviews, D15.1 also developed a long list of 'statements' (or positions in terms of argumentation analysis) for future use in exploration of information needs in policy makers and stakeholders. These can be used in further delineation of information needs.
What are common HBM mixture patterns in the European population?	So far, there are insufficient existing HBM mixture data available through the repository to address this question. Statistical scripts and approaches have been developed and tested on a simulated data set. These have been described in AD15.3 and D15.3. The scripts involve a combination of methods, both graphical and analytical, and combine alternative methods. In 2019 the scripts have been successfully applied to real HBM mixture data from the Flemish 'FLEHS' cohort under bilateral agreement. The results have been described in scientific manuscript that was recently submitted for publication. Meanwhile, similar analyses have been initiated for the German 'GerES' cohort and existing data from cohorts from Spain and Czech Republic are being lined up for subsequent analysis and for across country comparisons.
Can we identify hotspots or risk groups with high mixture exposures?	HBM4EU is running a survey of human internal exposure to mixtures of pesticides across five of our partner countries: Hungary, Czech Republic, Spain, Latvia and the Netherlands. Switzerland will also collect urine samples, with a slightly different design. This survey, entitled 'SPECIMEn', explores exposure to pesticides and focusses on residential areas or "hotspots" close to agricultural fields where pesticides are applied. The survey is designed to assess concomitant/combined exposure to multiple pesticides in hotspot and control areas using human biomonitoring. Details of the joint pesticide survey are described in AD15.7. The field work for this survey started in the fall of 2019 and is nearly completed. Urine samples will be collected in 50 parent-child pairs in hotspots (residences within 250 m of agricultural application of pesticides) and 50 parent-child pairs in control areas. Samples and questionnaires will be collected in a non-spraying and a spraying season. Samples will be analysed through pesticide suspect screening in conjunction to CGL Emerging Chemicals (WP16). These suspect screening approaches are built on non-selective analytical workflow and allow the qualitative monitoring of several hundred (up to several thousands) of exposure markers, including various pesticide classes under their parent or metabolite form. This approach will be used to gain insight into the occurrence of extended exposure patterns of pesticide-biomarkers, differences across the countries participating in SPECIMEn, differences between two seasons (spraying season with active application, and non-spraying season with no active application) and/or location (living close to agricultural areas or not). The results obtained will also contribute to the prioritisation of certain substances in terms of further exposure and risk assessment, and to possibly generate early warning information.
Which sources & pathways contribute most to HBM mixture values?	This policy question could not yet be addressed, but is being worked on in tasks 15.1 and 15.2. Results are expected in 2021.

Policy Question	Short Summary of Results
Which effect markers can we use to assess health risks of mixtures?	This question can be addressed in multiple ways: from the perspective of single chemical families and from the perspective beyond single chemical families. Obviously, the latter is more complex. Within HBM4EU, both perspectives are taken into consideration. Effect biomarkers for oxidative stress, i.e. 8OHdG and 8-isoprostane, are included as an add-on in the joint survey on pesticides 'SPECIMEn'. Further opportunities to include effect biomarkers in the joint survey on pesticides are being explored. Within Task 15.3, case studies are being conducted with the aim to identify methods for the prediction of mixture effects that can be used consistently for human health risk assessments and can inform biomonitoring strategies. In four of the five case studies conducted, in cooperation with WP13 and WP14, suggestions for effect biomarkers are expected as additional results of the case study. Additionally, activities on priority chemicals of specific chemical families may provide effect biomarkers of the combined group of chemicals in that family.
What action perspectives are available to reduce mixture levels?	In the exploration of policy needs for mixture risk governance, views on responsibilities and on criteria to guide risk reduction strategies varied considerably. Concrete action perspectives therefore, remain unarticulated. In the long list of 'statements' (or positions in terms of argumentation analysis), several pertain to action perspectives. These were mainly drawn and paraphrased from actual discourse on mixtures, and range from current legislation being sufficiently adequate to protect public health from mixture effects to concrete proposals how to better regulate exposure to mixtures and the associated health effects (see D15.1 for full list). While all these positions have been brought forward in discussions on action perspectives with respect to mixture risk governance, the overall picture as yet is anecdotal; it is unclear to what degree the various options have support in a wider constituency of experts, policy makers or the general public and stakeholders. A more systematic and broader consultation would be needed to gauge support for these (sometimes incompatible) alternative action perspectives to reduce mixture risks in the population. Such an activity is under consideration for the WP15 workshop on policy recommendations on risk assessment of chemical mixtures, which will be organised in conjunction with, among others, Pillar 1.

16 Prioritised substance group: Mycotoxins

<table>
<tr><th>Policy Question</th><th>Short Summary of Results</th></tr>
<tr><td>1. Are there validated and harmonised analytical methods to assess the target mycotoxins exposure?</td><td>An inventory has been made on biomarkers for deoxynivalenol (DON), fumonisin B1 (FB1) and aflatoxins and analytical methods, in WP9.1, deliverable report D9.5 “Prioritised list of biomarkers, matrices and analytical methods for the 2nd prioritisation round of substances”, v1.1 27th May 2019. An update version is foreseen for (June 2020). It has been decided that within HBM4EU only DON will be included in analysis of samples from aligned studies. Total DON (after deconjugation) in urine has been chosen as biomarker for exposure to DON and its derivatives (3/15-acetyl-DON, DON-3G). Harmonised methods and certified reference materials are not available. A list of 13 candidate laboratories for the chemical analysis of DON was elaborated after consulting the CGLs for suggestions. The list was included in the deliverable D9.6 ”Database of candidate laboratories for the 2nd prioritisation round of substances and update for the 1st round”. This list was used for the QA/QC program implementation in task 9.4. In order to identify expert laboratories, a questionnaire was sent to the candidate laboratories to gain more detailed information on validation status, experience and capacity to analyse HBM4EU samples. Based on these criteria, the QAU had selected 6 expert laboratories for mycotoxins to be included in a 3-round interlaboratory comparison study to assess comparability of analysis results.

However, one laboratory had to withdraw from participation (consequence of COVID-19). The third round is ongoing (samples were sent to the laboratories). Expected completion of the interlaboratory exercise is September 2020. The table below summarises the information about the QA/QC program for mycotoxins (total DON):

<table>
<tr><th>Substance group</th><th>Matrix</th><th>Compounds</th><th>Organiser</th><th>CM prep. & testing</th><th>ICI Round-1</th><th>ICI Round-2</th><th>ICI Round 3</th></tr>
<tr><td>Mycotoxins</td><td>urine</td><td>Total deoxynivalenol</td><td>RIKILT (WFSR)</td><td>RIKILT (WFSR)</td><td>6 labs (Jan-Feb 2020)</td><td>5 labs (Feb-June 2020)</td><td>5 labs (June-July 2020)</td></tr>
</table>
ICI = interlaboratory comparison investigation; consists of 3 rounds, after that a decision memo on comparability will be prepared (expected Sept 2020)</td></tr>
<tr><td>2. What are the current exposure levels of the European population to DON and FB1? Are there exposure data for other mycotoxins?</td><td>Since no data on mycotoxins exposure is available at HBM4EU database or repository, an initial literature search on Human Biomonitoring studies in Europe related to prioritised mycotoxins and also other mycotoxins (more abundant) is on course and expected to finish by July. Literature search was performed in Pubmed, Web of Science and Scopus with the keywords: Human AND Biomonitoring AND Mycotoxins, review papers, and EFSA reports. Until now 104 references were identified and included in Mycotoxins Biomonitoring Database.

Next, and within WP 10, task 10.4, a research protocol will be developed for the selected mycotoxins exposure (FB1 and DON and its glucoronides) and other main toxins, based in the mentioned literature search. The identification of existent data collections is being performed in order to determine which research questions can be explored with HBM data already available.</td></tr>
</table>

Policy Question	Short Summary of Results
	The draft research plan was presented and discussed in the WP 10 Meeting held in November 2019, in Paris and an update was recently presented in the virtual WP10 Workshop, organised in June 2020. A biomarker of DON exposure (urine total DON) is being included in the aligned studies for the adult population across Europe that is expected to also answer this question (Decision Memo on the implementation of Mycotoxins analysis in the aligned studies of task 8.1, by WP 8, submitted 2019 by CGL).
3. Does the exposure to mycotoxins differ among different population groups? Which are the main factors related with these differences (age, gender, settings, geographic localisation)?	Differences on mycotoxins exposure profiles as well as the geographic variations of FB1 and DON exposure will be ascertained either through the literature survey or through the new data obtained in the aligned studies.
4. Is there a time trend in human exposure to mycotoxins across Europe? Which are the identifiable factors associated with these trends (regulation related with food safety, climate change, others)?	This question will be addressed within the research protocol for mycotoxins within WP10, task 10.4.
5. Is the risk associated to human exposure to these mycotoxins characterised?	The assessment plan for mycotoxins was elaborated and described in the scope of task 5.3 (march 2020). New HBM data on mycotoxins will be used in the RA of mycotoxins, namely from i) bibliographic search, on course at WP10 (DON and FB1) and ii) through the performance of HBM4EU aligned studies (WP 8.1) in different European regions (only total DON). In the RA that will be performed, the general population (mainly exposed through food) will be the focus although occupational exposure may also be included if relevant exposure data is available. The HBM data considered in the scope of WP10 or the new HBM data obtained under the aligned study will be compared directly with the HBM-GV, if available. If not, a reverse dosimetry estimate comparing obtained data with available TDI values will be performed. The policy questions and activities mainly related to mycotoxins risk assessment were presented by CGL at the Joint Meeting HBM4EU and EFSA to exchange ideas and promote collaboration between both entities (February 2020).

Policy Question	Short Summary of Results
6. Are there exposure models and toxicokinetics data for mycotoxins and which are their limitations?	In Task 12.3, the parameterisation of the generic PBTK model developed in Task 12.1 for mycotoxins was performed. Specifically, a published human PBTK model for DON, with a focus on pharmacokinetic parameter values and methods used to estimate these values was taken into account. In addition, literature data, generally obtained experimentally (in vivo or in vitro) was also used for the estimation of parameter values. Regarding fumonisins, future efforts should be done to increase the knowledge of their toxicokinetics and consequently contributing to a better human risk assessment. Other mycotoxin PBPK model available include zearalenone and its metabolites. Summary of the human PBPK models for mycotoxins was reported in "Review of available models for the 2nd set of prioritised substances", Deliverable Report AD 12.8. As biological half-life values of mycotoxins DON and FB1 in humans were not found, data from half-life values in different biological media (plasma, liver, kidney, muscles and brain) of animals were reported in "Biological half-life and internal dosimetry of the 2nd set of priority compounds", Deliverable Report D12.7 (submitted June 2020).
7. Is it possible to set HBM guidance values for mycotoxins?	Based on literature survey and after consulting experts from WP 5, task 5.2 (from UBA, RIVM and ANSES) and the CGLs, the possibility of deriving a HBM guidance value for DON was identified and the work is currently being carried out. A "Decision Memo on the derivation of HBM-GVs for DON, provided by WP 5 (task 5.2), was submitted to the Management Board (June 2020) involving a close collaboration between RIVM, ANSES and INSA.
8. Which are the key events that determine the long-term health effects from low-dose continuous exposure to the target mycotoxins?	A focus group led by RIVM was organised for mycotoxins aiming at addressing this policy question. The first outcomes have been communicated to EFSA and was included in an interim report as AD13.6-2020 (AD13.6 - Answers from WP13 to exposure-health policy questions for the 2nd priority compounds; december 2019). Two draft Adverse Outcome Pathways (AOPs) for DON and FB1 were prepared last year, however, only for one of the mycotoxins will the development of an AOP be continued, since a link must be evident between the AO and adverse effects relevant for humans. A human effect, neural tube defects, has been identified after chronic exposure to FB1. No human health effects have been identified after chronic exposure to DON. Moreover, a biomarker of effect has been identified for FB1, which matches the AOP.
9. Which are the most reliable and informative AOP- based effect biomarkers for prioritised mycotoxins?	A working group (INSA and MU) addressed the effect biomarkers reported in HBM studies for the selected mycotoxins, FB1 and DON. The most relevant health outcomes were selected for each mycotoxin, based on the effects reported in several epidemiological and animal studies. In addition, the mechanisms of action that are deemed to underlie their toxicity (in vitro/ex vivo and in vivo studies) were considered, in an attempt to link the identified health outcomes to central molecular, cellular or tissue/organ key events. This knowledge is relevant to try to establish the molecular initiation event and the key events in order contribute to production of AOP for FB1 and hepatotoxicity, nephrotoxicity or carcinogenicity and for DON and immunotoxicy, reprotoxicity or endocrine disruption. These will liaise with the work performed in WP13. Increases in sphinganine and decrease in sphingosine levels and their ratio were identified as effect biomarkers in 8 HBM studies and they may be considered as specific markers linked to key cellular events of FB1 action (disruption of sphingolipids metabolism).

Policy Question	Short Summary of Results
	Other works have pointed to some endpoints and methodological approaches, e.g., gene expression or gene methylation analyses that deserve to be further explored to discover novel effect biomarkers. Regarding DON, a single study referring the use off effect biomarkers related to its immunotoxicity and autism was retrieved. The information was included in Deliverables 14.5 (Selection criteria and inventory of effect biomarkers for the 2nd set of substances) and 14.6 (Report on the state of development of Task 14.3)
10. Which research needs and gaps on Human Biomonitoring activities related to prioritised mycotoxins?	The work developed in the context of several WPs and tasks has evidenced several research needs and gaps on HBM studies that should be addressed in future studies, such as: o validation and harmonisation of analytical methods for mycotoxins, o standards and reference materials availability, o updated of HBM exposure data, to perform a more accurate RA and risk characterisation as well as to try to identify time trends and possible relation with climate changes (since exposure to some mycotoxins are expected to increase in Europe due to changes in climatic parameters), o determination of HBM GV and reference values for FB1, o further development of toxicokinetic models, o further epidemiological research on novel effect biomarkers for FB1 and validation of the existing ones; identification of effect biomarkers and AOP development for DON, o identification, characterisation and risk assessment of mycotoxin mixtures.

17 Prioritised substance group: PAHs and air pollutants

Policy Question	Short Summary of Results
Extract the main findings of the deliverable that answers (part) of the policy question, mention the deliverable	
1. What is the current exposure of the EU population to PAHs?	In WP12, exposure to PAHs was addressed using the data collected from available HBM data regarding 1-OH-pyrene. 1-OH-pyrene is a major metabolite of pyrene and is representative for the PAHs mixtures, while it is the metabolite that is commonly measured in the majority of PAH-related HBM studies. A PBTK model was parameterised and validated in Task 12.1 and it was coupled with the exposure reconstruction algorithms developed in Task 12.2. Based on the existing HBM data available at the moment, the median value of pyrene exposure ranges between 0.025 µg/kg_bw/d for non-smokers in Belgium to 0.240 µg/kg_bw/d for smokers in Netherlands. For most of the countries, median daily intake is around 0.050 µg/kg_bw/d, however, it has to be noted that, as described above, the bio samples had not been collected in the same year, while analyses were performed by different laboratories, thus, hampering the overall intercomparison; these estimates will be updated, upon the aligned study result will be available (AD12.5).
2. What is the current exposure of different occupational groups?	Exposure to the various occupational groups varies based on the specific activities of the related occupational sectors. The highest intake estimates were identified in soil remediation workers (in the range of 0.981 to 1.284 µg/kg_bw/d), followed by asphalt workers (0.093 to 0.325 µg/kg_bw/d) and workers in aluminum and rubber industry (0.035 to 0.100 µg/kg_bw/d). The lowest intake levels were identified to waste incinerator workers (0.004 to 0.104 µg/kg_bw/d), which is the only reported sector occupying both males and females. On the contrary, in all other sectors (soil remediation workers, asphalt workers, workers in aluminum and rubber industry) only males are being occupied and a differentiation on their intake results from their smoking habits, the time of their shift (pre shift, end of shift, post shift, next pre shift) and the age groups. The highest intake levels were related to soil remediation workers (1.284 µg/kg_bw/d) during the next pre shift, where pre shift and end of shift reported lower intakes (0.981 and 1.249 µg/kg_bw/d, respectively). For asphalt workers the highest intake was reported in the post shift and the specific age range of 35-52 (all workers were non-smokers). For workers in the aluminum and rubber industries, the lowest intake was reported for non-smokers (0.035 µg/kg_bw/d) comparing to smokers who exhibited a considerably higher intake (0.065 µg/kg_bw/d) (AD12.5).
3. Is there an association between air quality and human exposure to PAHs?	Dietary exposure dominates exposure to PAHs (contributing to almost 90 %) of daily intake, while the contribution of inhalation is lower (about 10%), except for the cases where significant sources of inhalation exposure such as the proximity to industrial hot spots, heavily trafficked roads, biomass emissions, as well as smoking; smokers have consistently higher exposure levels to pyrene, resulting to daily intake of between 0.015 to 0.150 µg/kg_bw/d. Regarding hot spots, it is expected that they result in higher pyrene concentrations in the range of 0.005 to 0.01 µg/kg_bw/d. (AD12.5)

Policy Question	Short Summary of Results
Extract the main findings of the deliverable that answers (part) of the policy question, mention the deliverable	
4. Does exposure differ between countries? Why?	The difference in intake levels among the various countries are mostly explained by the differences in dietary intake, which is the result of increased soil contamination and dietary patterns (frequency of eating smoked food) and to a smaller extent to difference in air pollution levels. More in detail, based on the available HBM data available so far, the highest intake levels were calculated in Netherlands (0.073 to 0.245 µg/kg_bw/d) followed by Germany (0.019 to 0.125 µg/kg_bw/d) and Greece (0.060 to 0.065 µg/kg_bw/d), Denmark (0.041 to 0.095 µg/kg_bw/d), Czech (0.053 µg/kg_bw/d), France (0.022 to 0.078 µg/kg_bw/d) and Italy (0.041 to 0.059 µg/kg_bw/d), Spain (0.035 µg/kg_bw/d) and Belgium (0.029 µg/kg_bw/d). The lowest intake levels were reported in Sweden (0.013 to 0.036 µg/kg_bw/d). It has to be noted that several exposure modifiers such as age, smoking status and exposure to secondhand smoke, as well as residential location have been identified as key factors affecting the overall intake levels. In Netherlands, Italy, France and Sweden the intake levels of smokers have been identified much higher compared to the ones of non-smokers (0.245 and 0.073 µg/kg_bw/d, 0.059 and 0.041 µg/kg_bw/d, 0.078 and 0.022 µg/kg_bw/d and 0.036 and 0.013 µg/kg_bw/d, respectively). In Germany the highest intake levels were reported for children of 5-8 years old, living near industrial hot spots (0.125 µg/kg_bw/d) while for children of the same ages living away from industrial hot spots the intake levels were much lower (0.064 µg/kg_bw/d). This is explained by the higher multimedia contamination in the area and the higher contribution to intake of both soil ingestion and ambient air inhalation. In Greece, living nearby areas with traffic congestion, the intake levels were higher than in urban areas free of traffic (0.065 and 0.060 µg/kg_bw/d, respectively). In Denmark the highest intake levels were reported for bus drivers of 27-60 years of age (0.095 µg/kg_bw/d) while the lowest ones were reported for people working in rural areas (0.041 µg/kg_bw/d) (AD12.5). However, the reason why differences are reported among the various countries will be further explored when the latest HBM data will be available and the statistical analysis in WP10 will have been completed.
5. Can we see a decline in exposure to the eight PAHs restricted under REACH?	Exposure to PAHs occurs through multiple pathways and routes. This also pertains for the 8 PAHs (benzo[a]pyrene, benzo[e]pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[j]fluoranthene, benzo[k]fluoranthene and dibenzo[a,h]anthracene) restricted under REACH. Restrictions from REACH are expected to affect the contribution of exposure related mainly to consumer products. It is also likely that the restriction of use will result in a reduction in the overall tonnage that will be reflected in the soil levels, which in turn will be reflected in the food chain and the dietary intake. However, to identify a potential decline, a trend analysis is required, which in turn requires the acquisition of the completion of the statistical analysis of existing data (from Tasks 10.3 and 10.4) and the collection of new data (Task 8.3: Targeted new field work with EU added value). At the moment there are not enough data to support this hypothesis.

Policy Question	Short Summary of Results
Extract the main findings of the deliverable that answers (part) of the policy question, mention the deliverable	
6. Can HBM4EU data inform the development of legislation specifically targeting exposure to PAHs through ambient air?	At the moment the EU Scientific Committee on Occupational Exposure Limits (SCOEL) has provided a biological guidance value (BGV) for PAH mixtures containing benzo[a]pyrene equal to 0.5 µg/L hydroxypyrene in urine. It has to be noted that the limit values recommended by SCOEL have not been implemented into legislation by the Member States. Based on the work that will be carried out in WP5, EU HBM-HBGV will be derived on the basis of toxicological studies. The values represent the concentration of a substance in human biological material below which there is no risk for adverse health effects and, consequently, no need for action. Hence, they are an important tool to easily assess whether the exposure of a population/subpopulation (e.g. reference values) is of health-relevance and whether policy actions are needed. These values will together with the result of WP10 be used also to address this research question. In addition, input will be provided from the work done in WP12, towards the association of the dose of toxic metabolites in the target tissue, with the observed HBM levels. In addition, work on exposure reconstruction of PAHs has indicated that most of exposure to PAHs comes from dietary sources rather than ambient air pollution, which is contributing for almost 10% of the overall exposure to diet (AD12.5).

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

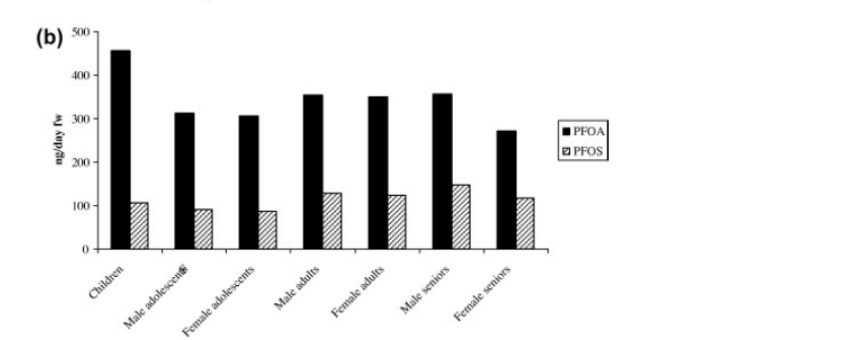
Policy Question	Short Summary of Results
1. What is the current exposure of the EU population to PFASs and do they exceed Guidance values (reference and HBM values), where available?	To examine the current exposure of the EU population and the respective health effects is task of several work packages 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 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.

Policy Question	Short Summary of Results
	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, 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.

Policy Question	Short Summary of Results
	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 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.

Policy Question	Short Summary of Results
	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, 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, 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.

Policy Question	Short Summary of Results
	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.

Policy Question	Short Summary of Results
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 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 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.

Policy Question	Short Summary of Results
11. How can mixture effects of environmental and human PFASs mixtures present to date be estimated?	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 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.

Policy Question	Short Summary of Results
	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-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.

Policy Question	Short Summary of Results
	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 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.

19 Prioritised substance group: Pesticides

Policy Question	Short Summary of Results
1. Which are the most suitable methods and biomarkers of exposure?	In WP9 a prioritised list of biomarkers, matrices, and analytical methods has been elaborated (D9.5). The list present 9 suitable biomarkers for the category B pesticides (chlorpyrifos, glyphosate and pyrethroids and 4 biomarkers for the category C pesticides (dimethoate and fipronil). Urine is the selected matrix for all the compounds except fipronil for which serum is the preferred matrix. Glyphosate is excreted both unchanged and as metabolites in urine. One urinary metabolite, aminomethylphosphonic acid (AMPA), is also the main environmental degradation product. Since AMPA and glyphosate has similar toxic profile, it is advised to measure both glyphosate and AMPA in urine as biomarker for the total glyphosate exposure. Chlorpyrifos is metabolised to TCPy (3,5,6-trichloro-2-pyridinol) and two unspecific diethyl phosphate biomarkers (DEP and DETP) which are all excreted in urine. TCPy is the most specific biomarker for chlorpyrifos exposure although two other pesticides, chlorpyrifos-methyl and triclopyr, are also metabolised to TCPy. The biomarkers for pyrethroids include a group-specific biomarker, 3PBA (3-Phenoxybenzoic acid), representing the combined exposure to many pyrethroids, 5 semi-specific biomarkers representing exposure to two-five pyrethroids, and a specific biomarker for deltamethrin exposure. Two of the biomarkers have not been measured in large population studies, while the remaining pyrethroid biomarkers have been developed and included in different large biomonitoring programs. Dimethoate is rapidly metabolised to unspecific dimethyl phosphates (DMP, DMTP, and DMDTP) which are excreted in urine. No suitable specific biomarker for dimethoate was available. The sum of the molar urinary concentrations of the three dimethyl phosphates will provide an estimate of dimethoate and other methylated organophosphate pesticides. By also including the three diethyl phosphates (DEP, DETP, and DEDTP) an estimate of the total exposure to organophosphates (including dimethoate and chlorpyrifos) will be achieved. All six metabolites (dialkyl phosphates, DAPs) are normally analysed in the same analytical run. Fipronil is mainly metabolised to fipronil sulphone which can be measured in serum/plasma. This biomarker has only been used in few studies and most of these are animal studies. LC-MS/MS was evaluated to be the most suitable analytical method for analysing all the biomarkers except for the pyrethroid biomarkers, CIF3CA (Cis-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylic acid) and trans-CDCA (trans-chrysanthemumdicarboxylic acid) for which GC-MS/MS and GC-HRMS, respectively, were assessed to be more suitable. No information was found for biomarkers for the glyphosate co-formulant Polyethoxylated tallow amine (POEA) or the pyrethroid co-formulant piperonyl butoxide (PBO) and therefor no methods could be selected. A database of candidate laboratories for analysing the 2nd round of substances has been elaborated (D9.6) and 36 laboratories were listed as candidates for analysing pesticides. Regarding the ICI/EQUAS programme for the 2nd round of substances, it was decided that that the approach used for the 1st set of substances would not be feasible due to time limitations.

Policy Question	Short Summary of Results
	Thus, a shortened ICI/EQUAS scheme focused on the substances foreseen to be analysed in the aligned studies was established. For the pesticides, 4 expert laboratories were selected for analysing biomarkers for pyrethroids (cis-DBCA, cis-DCCA, trans-DCCA, 3-PBA, 4-F-3-PBA, ClF3CA), glyphosate (glyphosate, AMPA), and chlorpyrifos (TCPy). These labs are included in the ICI programme planned to be completed by mid-2020
2. What are the current exposure levels of the EU population to the prioritised pesticides: pyrethroids, chlorpyrifos and dimethoate, glyphosate (in combination with polyethoxylated tallow amine (POEA)), and fipronil and do the exposure levels differ between countries? **3. What are the main dietary sources of exposure across the member states?** **4. What are other potential sources and pathways of exposure?** **5. What are exposure levels among occupationally exposed workers?**	WP10 has developed a general and a substance-specific statistical analysis plan including variables, which are needed for the statistical analyses to address research questions for the pesticides on general exposure level including identification of high exposure subgroups, time trends including potential impact of regulation, geographic comparisons, and exposure determinants (D10.5). A comprehensive review of the existing HBM-studies on the prioritised pesticides (including information on occupational exposures and other exposure determinants) performed within the HBM4EU associated countries is in progress in the scope of WP10. Overall, there are relatively few studies, several were performed before 2010, they are not EU-wide, and few studies address occupational exposures. Besides, analyses of the main dietary and non-dietary sources of exposure will be analysed using existing HBM-data from selected data-collections. A protocol for this work is in preparation in WP10. For dietary exposure, an attempt to analyse/model HBM data in relation to data on pesticide residues in food will be performed with the purpose to compare and complement exposure assessments performed by EFSA. In this regard, a thorough revision of the main sources and pathways of exposure to pesticides at international level was performed within WP7 (Task 7.3), in order to develop a specific questionnaire to characterise the exposure to these substances in the study population of the aligned studies (see below). This questionnaire is aimed at supporting the identification of the main sources and pathways of human exposure to pesticides across the member states related to residential environment and home exposures, dietary habits, lifestyle and occupation (D7.6). Further, a gap analysis was performed in WP7 to get an overview of the number of studies of the 2nd round of priority substances within the participating countries based on a questionnaire distributed in 2018 (D7.5). A total of 26 studies (conducted or initiated/ongoing) reported to analyse pesticides. Most of these studies were carried out in the western European-defined region followed by the North and South regions and Israel. No studies on pesticides were reported from the East region. Children were included in 12 of the studies while adults were included in 18, adolescents in 4, and elderly in 2 studies. Of these studies, 17 reported to have samples representative at national level (7 for children, 2 for adolescents, 8 for adults, and none for elderly). Within WP10, metadata for existing HBM data collections are integrated in IPCHEM, for which 16 data collections indicated to have HBM data on pesticides. From those, 9 data collections shared harmonised aggregated data for pesticides. These data can be accessed via the HBM4EU internal webpages. These data were included and visualised by exposure distributions in D10.6. To obtain a better EU coverage for HBM exposure data a sampling framework has been developed in WP8 by including 33 studies (known as the aligned studies) from 21 countries representing north, south, east, and west Europe. For the pesticides, biobanked urine samples from children aged 6-11 years from eight different countries (Norway, Denmark, Hungary, Slovenia, Italy, France, Germany, and the Netherlands) are planned to be analysed for glyphosate, chlorpyrifos, and pyrethroid biomarkers (D8.7 and D8.8). In the SPECIMEn study performed in WP15 (see AD15.7 for detailed description), urine samples are collected during the spraying and non-spraying season from children and their guardians living close (within 250 m) to agricultural fields treated with pesticides as well as from urban control populations, in five EU countries.

Policy Question	Short Summary of Results
	These urine samples will be analysed by suspect screening approach developed under WP16, aiming to qualitatively detect an extended range (i.e. several hundreds to thousands) of pesticide related markers of exposure through a developed harmonised analytical workflow (from sample preparation, LC-HRMS data acquisition to data processing and reporting) applied by five laboratories/countries (FR, NL, SP, CZ, DE). The results will gain insight in the feasibility of this analytical approach, and information on differences in detection frequencies of pesticides and exposure patterns between the included countries and subpopulations. From this provided picture of the reality of human internal exposure, these results will contribute to further rationale and prioritisation with regard to certain markers for which more quantitative and targeted methods will appears necessary and/or for which the inclusion in future HBM program will appears relevant.
6. Are the exposure levels of health-relevance/concern for vulnerable groups (infants, children and pregnant women) or high exposure population groups (e.g., occupational exposure)? **7. How can cumulative risks of pesticide mixtures on sensitive health outcomes be assessed and integrated in regulation?** **8. Is it possible to establish EU wide accepted HBM guidance values for the pesticides that takes into account potential mixture effects and evidence from epidemiological studies?** **9. How can HBM data from HBM4EU feed into prioritisation of the pesticides for risk assessments and regulatory decision-making?**	Within WP13 and WP14, comprehensive literature reviews on health effects and toxic mechanisms have been performed. The primary aim of the reviews was to assess HBM exposure levels associated with adverse health outcomes taking into account vulnerable exposure windows (pregnant women and children), and to identify the most important mechanisms and potential adverse outcome pathways (AOPs) and suitable effect biomarkers for the health outcomes of highest concern (D13.5 and D14.5). For chlorpyrifos, dimethoate, fipronil, pyrethroids and glyphosate, several classical and novel effect biomarkers for a range of health outcomes (cardiometabolic health, the immune system, pregnancy and reproductive outcomes, neurodevelopment and cancer) has been inventoried (D14.5). In the scope of WP13, the text mining tool (AOP-helpFinder) developed at INSERM was applied to the prioritised pesticides to identify linkages with AOP events existing in the AOP wiki database. This tool screen automatically abstracts from the PubMed database. The results have been collected, structured and presented in a webserver named AOP4EUpest (AOP4EUpest: Mapping of pesticides in Adverse Outcome Pathways using a text mining tool, published in Bioinformatics (Jornod et al., 2020)). A review publication entitled "Reproductive health risks associated with environmental and occupational exposure to pesticides" has been initiated. Besides, associations between HBM data and health outcomes are being analysed within some of the aligned study cohorts, e.g., between pesticide exposure in pregnancy and child health outcomes in the Odense Child Cohort and between glyphosate and DNA damage and immunological effects in the FLEHS IV cohort. Two joint WP13/WP14 review publications are in process in which epidemiological evidence on 1) developmental neurotoxicity (DNT) and 2) reproductive/endocrine disturbances will be integrated with mechanistic knowledge to identify plausible toxic mechanisms, potential AOPs, and suggestion of effect biomarkers. The AOP4EUpest is used to help identifying AOP events. If possible, meta-analyses will be performed but is hampered by considerable differences in methods used for assessing the outcomes (e.g., neurodevelopment). Since chlorpyrifos/chlorpyrifos-methyl did not get renewal of authorisation (expired 31 January 2020), it was decided to focus on pyrethroids in these reviews. Based on examples from the 1st set of substances it was concluded in WP5 that HBM would be particularly important for performing risk assessment (RA) for substances, for which several exposure routes may contribute to the body burden and the health effects. This is exactly the case for the pesticides, especially for pyrethroids used both as biocides and as agricultural pesticides. One of the major challenges mentioned was the, most often, limited data on toxicokinetic and lack of specific and/or sensitive analytical biomarker methods (D5.5). It is planned in WP5 to derive HBM guidance values (HBM-GVs) for deltamethrin and one other pyrethroid. These values will be compared with urinary concentrations obtained from the aligned studies in 2020-21 and with concentrations reported from existing data-collections in the EU inclusive studies on adverse health outcomes collected in WP13 and WP14.

Policy Question	Short Summary of Results
	To enable translation of HBM data into external exposure levels for comparison with e.g., ADI values, information on toxicokinetic properties is needed. This issue is addressed in WP12 using toxicokinetic modelling to link an external exposure to an internal dosimetry in humans (e.g., concentration in blood, urine or in tissues) by describing the process of absorption, distribution, metabolism and excretion (ADME) that undergoes a substance in living organisms. A class of toxicokinetic models, the physiologically based pharmacokinetic (PBPK) models, bases the description on the ADME processes on the physiology and the anatomy of individuals, and the biochemistry of the compounds. A comprehensive review of human PBTK models available for the HBM4EU 2nd set of compounds, including the prioritised pesticides, has been performed (AD12.8). Most models concern adults while toxicokinetic data on sensitive populations (pregnant women, foetuses or children) are still missing. Existing PBTK models for humans have been identified for four pyrethroids: deltamethrin (type II pyrethroids), permethrin (type I pyrethroids), cypermethrin (type II pyrethroids) and cyfluthrin (type II pyrethroids). Interestingly, one of the models for deltamethrin predicted a considerably higher brain concentration in humans than rats due to an almost six-fold higher cardiac output to the brain in humans. A generic model for pyrethroids has also been proposed as a tool to interpret the combined exposure to pyrethroids reflected by non-specific urinary biomarkers. Several PBTK models were also identified for chlorpyrifos and other organophosphate insecticides while no specific PBPK-models for neither humans or animal species were identified for glyphosate or the co-formulant Polyethoxylated tallow amine (POEA) or for fipronil. Some of the PBPK models will be parameterised and used for forward or reverse dosimetry to either translate HBM data for the pesticides into external exposure doses for comparison with established guidance values for risk assessment (e.g., ADI) or to simulate the biologically effective dose of the compounds (AD12.10). However, most HBM studies on adverse health effects related to pyrethroid exposure are based on the group specific urinary metabolite 3PBA (3-phenoxybenzoic acid) reflecting the combined exposure to pyrethroids. The reason is that the detection frequency of more specific metabolites is typically very low because different pyrethroids are used alternately and they have short biological half-lives. Thus, besides comparing HBM concentrations of specific metabolites with the HBM-GV derived in WP5, it will be investigated how group-specific biomarkers, such as 3PBA, can be translated into external exposure levels and integrated in assessment of cumulative risks of pesticide mixtures. Another major activity related to cumulative risk assessment is the investigation of exposure to mixtures of pesticides among residents living close to pesticide treated areas in the SPECIMEn study performed in WP15 (results are further described under the Substance group: mixture).

20 Prioritised substance group: Phthalates & DINCH

Please note: Not all policy questions are listed – only those which have been addressed until now with notably results. You can find all 13 PQs in the scoping document.

Policy Question	Short Summary of Results
Exposure characteristics	
1. Which are the most sensitive, reliable and cost-effective methods and biomarkers to measure phthalates and Hexamoll® DINCH®?	In WP9 a prioritised list with most suitable biomarkers, matrices and analytical methods has been elaborated. In total 26 suitable biomarkers representing exposure to 14 parental compounds were selected. Two methods have been evaluated as being suitable to measure the metabolites: GC-MS-MS for measuring DPHP metabolites only and LC-MS-MS for all other biomarkers. Urine has been selected as matrix of choice for all compounds. No information has been found for DiPeP, DHNUP and DMEP. Hence, no methods or biomarkers could be selected (see D9.2). Furthermore, a final list of the parameters that will be included in the ICI/EQUAS 2018 has been elaborated in substance specific working groups based on the existence of solid and reliable analytical methods and the availability of reference material. The following compounds have been selected to be obligatory: DEP, BBzP, DiBP, DnBP, DCHP, DnPeP, DEHP, DnOP, DiNP, DiDP, DINCH. Additionally, DMP and DPHP can be included on a voluntary basis. In WP 9.3 “Development of new methods”, a feasibility study was conducted that identified new, valuable urinary exposure biomarkers for EU-labelled, reprotoxic phthalates currently not covered in HBM analytical methods (Cat C phthalates): Di-isopentyl phthalate (DiPeP), Di-C7-11-(linear and branched)alkyl phthalate (DHNUP), Di-n-hexyl phthalate (DnHexP) and Di-(methoxyethyl) phthalate (DMOP). Biomarkers for these phthalates will soon be implemented in a new phthalate multi-method and tested in WP9.3 with a small round robin test. A Quality Assurance/ Quality Control Programme was implemented in order to establish a European database of candidate laboratories that are equally qualified for exposure biomarker analysis within HBM4EU. For this an interlaboratory comparison investigation/external quality assurance scheme (ICI/EQUAS) scheme and evaluation criteria were developed (see Deliverable 9.4). Throughout the ICI/EQUAS exercise, in combination with training and knowledge exchange, a substantial increase in capable laboratories being able to analyse DINCH/phthalates within HBM4EU was achieved: Aafter the 4th round ICI/EQUAS a total of 20 laboratories from 14 countries could be identified, that successfully participated for the analysis of phthalates biomarkers. More than half of the laboratories qualified for the analysis of DEHP, DEP, DiBP, DnBP and BBzP biomarkers (≥11/ 20). For the Cat B and C phthalates also an increasing number of approved laboratories was achieved, ranging from six to ten laboratories. For DINCH metabolites, 8 laboratories from 8 countries did successfully participate of which all qualified for the analysis of OH-MINCH and almost all for cx-MINCH (7/8). The most current list of candidate laboratories can be found on the HBM4EU online library (https://www.hbm4eu.eu/online-library/).

Policy Question	Short Summary of Results
2. What is the extent of the current exposure of the EU population to the 16 phthalates (Cat A, B and C) and their substitute Hexamoll® DINCH®? **&** **3. Do the exposure levels differ significantly between the countries?** **&** **5. What are the high exposure groups? (Is there a statistically significant and toxicological relevant difference in mean concentration between adults and children? […] between occupational exposed and non-exposed adults? […] between male and female?**	In preparation for answering the policy questions, WP10 has developed a general and a substance-specific statistical analysis plan for phthalates and DINCH. Variables, which are needed for the statistical analyses to address substance-specific research questions on general exposure levels, time trends, geographic comparisons, and exposure determinants and reference values were defined. A requirement for the statistical analyses is the sharing of data as laid down in the Data Management Plan. The HBM4EU DMP gives details on the procedures that ensure that data are transferred and used in a secure setting; the use of the data is compliant with ethical and legal requirements, and that the use of data gathered within and before HBM4EU is done in agreement with the Data Contact Point. (see Revised DMP, D10.7). In WP7.1 a gap analysis has been carried out to get an overview how many studies of the priority substances, including phthalates and DINCH are available within the participating countries and has been summarised in a report (see D7.1). 42 studies in 12 different countries have been conducted or are initiated/ongoing, with measurements of phthalates and/or DINCH exposure over all age groups (New-borns, Children, Adolescents, Adults and Elderly). In general, most studies on this substance group have been carried out in the Northern or Eastern European-defined regions. 32 of the 42 studies reported to have biobanked samples and 6 of these studies are representative at national level. For the phthalate and DINCH substance group, most of the studies reported were with children and these studies were mostly conducted in Western Europe. Data owners/providers of 23 different studies on phthalates and DINCH have already provided their metadata to the HBM4EU repository and the data will continuously be included. Up to now, metadata of 49 different datasets, which measured phthalates from 20 different countries are included into IPCHEM. As these existing data collections are heterogeneous in terms of sampling time, biomarkes, matrices and study populations, WP10 developed an R-script in order to be able to obtain harmonised aggregated data of these different data sets. 27 data collections from 12 different countries shared harmonised aggregated data for phthalates and 1 data set for DINCH (German ESB) could be harmonised based on this script. Exposure distributions of the obtained merged harmonised aggregated data output files was visualised by using box plots based on different percentiles (P5, 10, 25, 50, 75, 90, 95). For more details, please see D10.6. Gaps in EU-representative data on exposure to phthalates and DINCH (e.g. missing regions and/ or exposure biomarkers) are being filled in by targeted analyses of biobanked samples and/or by studies of planned or ongoing HBM studies in the participating countries with 50% of HBM4EU funding. A sampling frame to obtain EU wide coverage with recent HBM exposure data was developed in WP8 (See D8.1). For phthalates and DINCH biobanked urinary samples will be analysed, already analysed data shared and new data will be collected in children aged 6-11 years and for teenagers aged 12-19 years for all geographical regions. For the Northern region Norway and Sweden will analyse biobanked samples from a national study, whereas Denmark will collect new samples in a regional study. In the Eastern region, Hungary and Slovakia will analyse biobanked samples and collect new samples in national studies, whereas Poland contribute with biobanked samples from a regional study. For the Southern region, Slovenia, Greece and Italy will collect new samples in national and regional (Italy) studies and Spain contribute with biobanked samples from a national study.

Policy Question	Short Summary of Results
	In the Western region, France will contribute with the analysis of biobanked samples from a national study and Germany will share data from a national study. The Netherland will collect new samples from the HBM4EU Specimen study to be analysed for phthalates and DINCH metabolites in the alignment of studies and Belgium will contribute with new samples collected from a regional study (see D8.4). Until now, datasets on phthalates and DINCH metabolites in teenagers have been received from Sweden, Spain, Belgium and Germany and for children from Denmark (see: https://www.hbm4eu.eu/online-library/). In WP10 different research protocols have been developed to investigate the difference in exposure to phthalates and DINCH between European countries; to identify high exposure groups as well as identify exposure determinants from existing data collection as well as from the new data collections done in Task 8.1 “Alignment of studies”. More information can be found in the updated Statistical Analysis Plan (D10.10) and on in the WP10 internal webpages of the HBM4EU website.
4. What are the main sources of exposure and the reasons for differences in exposure (different regulations in different countries) to phthalates and Hexamoll® DINCH®?	WP7 has developed a concept for a study protocol for recruitment and sampling to ensure harmonised recruitment, sampling and questionnaire implementation. This harmonised procedure aims at obtaining comparable results across countries involved in the HBM4EU targeted studies. A substance-specific questionnaire for phthalates/DINCH was developed to collect all the necessary information concerning individual characteristics of the participants (sociodemographic, dietary, occupational, lifestyle, environmental and health factors) with the aim to characterise as well as identify possible sources and routes of exposures to these substances (see D7.3 and D7.6). In WP 10.4 a protocol has been developed that will investigate exposure determinants on existing data sets on phthalate exposure for several age groups and different phthalates metabolites (see: https://www.hbm4eu.eu/online-library/). In addition, the 2nd occupational study will investigate by measuring urinary phthalates metabolites in workers of companies from 10 different countries, among others, what are the most relevant compounds in e-waste processing. Thereby, giving insights in important occupational exposure sources in the recycling sector.
Monitoring the success of existing policy actions and assessing the needs for further regulation	
6. Are there different time trends for unregulated (DEP, DMP, DCHP, DPHP) and regulated phthalates (DEHP, BBzP, DnBP, DiBP, DinP, DnOP) and Hexamoll® DINCH®?	In WP 8.2 will evaluate time trends for DINCH and phthalates in one or to age-groups and 4 European geographical areas by comparing three different time points (D8.4). For the first time point (2006-2010) already published exposure data will be used, for the second time point (2011-2013) new analysis of DEMOCOPHES samples will be conducted and for the third time point (2014-2020) data from the alignment of studies will be used. So far, no published information on DINCH exposure in children were found for the first time point, and only little information for adults. It will be explored whether biobanked samples can be accessed to gain more information on DINCH exposure for the first time point as several biobanks from all 4 geographical regions exist, even though not for each age group of interest. Information for several phthalate metabolites are available in the literature. For the second time point, DEMOCOPHES samples will be analysed. Up to now, already 9 partners have replied positive to perform a new analysis with 50% cofounding from 3 geographical regions. The analysis of DINCH in these samples is already agreed upon. For the detailed description of planned measurements for the third time point, please see above and D8.4.

Policy Question	Short Summary of Results
7. How effective have the different mitigation steps and regulations been for phthalates?	A protocol for examination of the temporal trends of phthalate exposure has been elaborated (WP10, task 10.4). Germany and Denmark have available and suitable data collections to be used in the time trend analysis (cross-sectional studies with repeated measurement design). Since there is only very few European studies with such a design data on young adults from two countries will be used to compare time trends in Cat A and Cat B phthalates and DINCH between 2000 and today. Based on the analysis a first picture of the temporal trends of exposure to regulated and unregulated phthalates will be drawn. In addition, a protocol under WP 8 has been developed in order to evaluate time trends for DINCH and phthalates (see results for PQ 6). On that account, it can be monitored how successful the existing policy actions have been and assessed where there are needs for further regulation.
Impact on human health	
8. Is the exposure to phthalates and their substitutes of health relevance for the general population and vulnerable groups? **What part of the population has exposure levels exceeding the HBM guidance values or TDI?** **&** **9. Is the health-relevance dependent on age or gender?**	In WP 5.3 an exercise was conducted for several phthalates for the general population if risk assessments could be improved by Human Biomonitoring data, and what the strengths and limitations are in using HBM in RA (D.5.5). For DEHP and the alternative plasticizer DINCH, RCRs were calculated based on metabolite concentrations in the DEMOCOPHES study using the HBM-GVs derived in task 5.2 and these were compared to RCR calculated in the restriction dossier from ECHA and the Danish EPA, 2016. As a result, RCR were in generally higher for children and lower for mothers when using the HBM-GVs. Finally, employing HBM data to monitor the implementation and effectiveness of the REACH restriction, and for studying time trends of the four restricted phthalates as well as the substitute phthalates are discussed. The work concerning the occupational population covered DiNP, DiDP and DPHP, because their use has not been extensively restricted in the occupational field and they are widely used in plastic product manufacturing. The calculated RCRs were well below one for DiNP and DiDP, based on a rough Biomonitoring Equivalents (BE) approach. The urinary concentration of the DPHP metabolite OH-MPHP is roughly 40x lower than the provisional EU HBM-GV of 0.9 mg/L, indicating a low occupational risk for this individual phthalate, based on conservative assumptions. Population exposure to phthalates and their substitutes is of outermost relevance for both the general population and especially vulnerable groups including pregnant women, children and adolescents. This is due to three main reasons: 1) Exposure is ubiquitous and virtually all the population is exposed to phthalate metabolites in a daily basis; 2) Recent systematic reviews are showing that current levels of exposure to specific phthalate families are associated with reproductive, neurodevelopmental and other health endpoints[8]; 3)

[8] Ribeiro CM, Beserra BTS, Silva NG, Lima CL, Rocha PRS, Coelho MS, Neves FAR, Amato AA. Exposure to endocrine-disrupting chemicals and anthropometric measures obesity: a systematic review and meta-analysis. BMJ Open. 2020;10(6):e033509. doi: 10.1136/bmjopen-2019-033509.
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Policy Question	Short Summary of Results
	The described exposure-health associations in the population are supported by toxicological knowledge. WP 14 conducted an extensive literature review in order to have a detailed overview of existing biomarkers of effect for phthalates (D14.2). This included both, long established “traditional” effect biomarkers and less studied “novel” biomarkers of effect. Several effect biomarkers of different health outcomes, such as cancer, effects on reproduction, neurobehavioral changes, endocrine disruption, allergy or effects on immune system, allergy and cardiovascular or metabolic endpoints has been inventoried. For phthalates a strategy for the selection of effect biomarkers for specific chemicals, health outcomes and window of exposure (i.e., biomarkers of reproductive effects associated with phthalate exposure in children/adolescents), as a proof of concept has been conducted jointly between WP 14 and WP 13 and published (doi: https://doi.org/10.1016/j.envres.2019.05.013). Here, an overview of effect biomarkers for reproductive toxicity are presented that are substantiated with mechanistic information (e.g. AOPs). WP 13 did give a detailed overview of the available knowledge on AOPs for phthalates (D13.4) and subsequently summarised key knowledge gaps emerging from previous research. Specific activities, studies or other relevant steps to fill the missing information was proposed (D13.5). As a result, the following target receptors are proposed that may initiate events leading, among others to impaired male and female fertility: PPARα, PPARγ and GR. In a second step a thorough process in prioritising the best suited biomarkers of effect to be utilised in human epidemiological studies were conducted (D14.3). For phthalates several novel and traditional biomarkers of effect are proposed to be implemented in the HBM4EU aligned studies (WP8) for the following endpoints measured in children and adolescents: neurodevelopment, asthma and allergy, sexual maturation, testicular function and metabolism and BMI. This will serve as proof of principle to examine that the implementation of specific effect biomarkers will complement the interpretation of exposure biomarker measurements and thereby support the weight of evidence of exposure health-relationships. WP12 has developed and improved a methodology for exposure reconstruction to deliver external exposure estimates from available (existing) HBM data (see AD12.6). Aggregated HBM data from 13 different countries for different age groups including young children, young adults. seniors and (pregnant) mothers were used for the assessment. Daily intake estimates could be established for DEHP, DINP, BBzP, DnBP and DINCH. For DEHP, DINP and DnBP, in most of the studies included mean daily intakes were close or above 1 µg/kg bw/ d, whereas for DINCH estimates were lower and for BBzP markedly lower. These values are below EFSA’s TDIs for the respective single compounds (DnBP = 10; BBzP = 500; DEHP = 50; DINP and DIDP = 150 µg/kg bw/d) (see AD12.5). Within WP10 research protocols have been developed to investigate what proportion of the population from children and adolescents (data obtained via Task 8.1 “Alingment of studies”) as well as which population from exisiting data collection does exceed HBM-GVs and also whether the mean concentration values differ with age or gender. More information can be found in the updated Statistical Analysis Plan (D10.10) and on in the WP10 internal webpages of the HBM4EU website.

Radke EG, Glenn BS, Braun JM, Cooper GS. Phthalate exposure and female reproductive and developmental outcomes: a systematic review of the human epidemiological evidence. Environ Int. 2019;130:104580. doi: 10.1016/j.envint.2019.02.003.

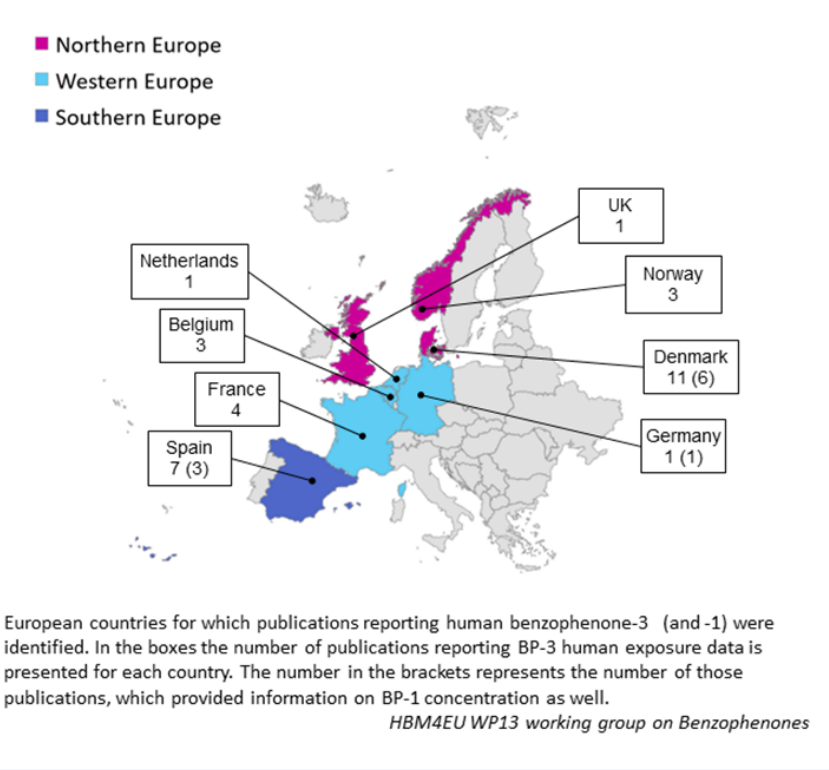
Policy Question	Short Summary of Results
10. Can EU-wide accepted HBM guidance values be derived for single substances and for the additively acting phthalates?	WP5.1 elaborated a concept document on the strategy for the derivation of health-based guidance values for the general population (HBM-GVGenPop) and for occupationally exposed adults (HBM-GVWorkers), thereby referring to the statement of the German Human Biomonitoring Commission on the basic principles for the derivation of HBM values as well as to the statement of the National Public Agency for Food, Environment and Occupational Health and Safety the occupational aspect of HBM values derivation. The strategy was applied for DINCH and DEHP metabolites and consolidated HBM guidance values were derived for DINCH & DEHP. For DEHP HBM-GVsGenPop of the sum of the metabolites 5-oxo-MEHP and 5-OH-MEHP or alternatively the sum of 5cx-MEPP and 5-OH-MEHP has been derived for adults and children (see D5.2): Σ [5-oxo-MEHP and 5-OH-MEHP] in urine: Children (6 - 13 y): 340 µg/L Adults: 500 µg/L Σ [5cx -MEPP and 5-OH-MEHP] in urine: Children (6 - 13 y): 380 µg/L Adults: 570 µg/L. For DINCH HBM-GVsGenPop of the sum of the metabolites OH-MINCH and cx-MINCH has been derived for adults and children (see D5.2): Σ [OH-MINCH and cx-MINCH] in urine: Children: 3 µg/L Adults: 4.5 µg/L. In addition, for the exposure in the workplace an HBM-GVWorkers of 0.62 mg/L has been derived for the metabolite 5cx-MEPP in urine at the end of the work shift. Currently, the derivation of HBM-GV for the following phthalates is being published in a peer-reviewed journal: DPHP, DnBP, DiBP and BBzP. In addition, the concept for a harmonised HBM-GV derivation developed under HBM4EU has been submitted in a peer-reviewed journal and is currently under review- HBM-GV are derived on the basis of toxicological studies. The values represent the concentration of a substance in human biological material below which there is no risk for adverse health effects and, consequently, no need for action. Hence, they are an important tool to easily assess whether the exposure of a population/subpopulation (e.g. reference values) is of health-relevance and whether policy actions are needed. Additionally, for each HBM-GV an level of confidence (LoC) is given, reflecting the underlying the uncertainties in the underlying database. Thereby, HBM-GVs can also help to identify research needs. These values will together with the result of WP10 feed also into addressing the research question 8.

Policy Question	Short Summary of Results
11. How can cumulative risks of phthalates and other anti-androgenic substances be assessed for their health relevance? Are their additive effects relevant for regulation?	WP15 has developed case studies of mixture effects of pollutants within the HBM4EU project. The case studies should focus on exposures and on health endpoints of concern and include different approaches from both toxicology and epidemiology. Phthalates are included in a case study that will evaluate the potential for a human health risk due to the present exposure to complex mixtures of 'anti-androgenic' chemicals based on our current knowledge. The group of anti-androgenic chemicals is expected to be very diverse, including phthalates, phenolic substances, certain pesticides and pharmaceuticals such as analgesics. Firstly, the known and widespread 'antiandrogenic' chemicals and drugs which humans are exposed to will be identified and gathered through existing literature on 'antiandrogenic' mixtures. Afterwards, hazard data from available sources including in vitro assays (AR reporter gene assay and the H295R steroidogenesis assay), ex vivo assays and, if available, in vivo data will be collected. Subsequently, relevant exposure data from available sources (human exposure levels, Cmax values for drugs, µM internal exposure levels) will be compiled. The Hazard Index approach will be employed and hazard quotients will be calculated. The analysis will be refined in the light of data on the likelihood of co-exposures. The work will be linked to investigations of antiandrogenic effects in placenta extracts which is ongoing in WP14 (in AR reporter gene assay and H295R steroidogenesis assay). In addition, a joint work between WP5.2 and WP15.3 has been started in which a mixture risk assessment of five selected phthalates will be conducted.
Usage of HBM4EU results for policy making	
12. How can HBM4EU results feed into the regulatory decisions of ECHA and EFSA?	HBM guidance values for phthalates and DINCH (WP5) are a useful tool to determine if a concern to human health might exist for the exposure to phthalates and DINCH and therefore measures, e.g. policy actions need to be taken. In order to ensure a good interaction between Task 5.2 and the EU Policy Board it is now foreseen to include the EU Policy Board into the consultation process for the different HBM-GVs to be derived to allow for input during the consultation period. The investigation of exposure sources by the statistical analysis group (WP10) can help identifying major exposure sources for each substance in the group. Hence, specific risk reduction measures can be implemented in policies to ensure safe exposure from major exposure sources, e.g. food and food contact materials or by restriction of the use of substances in articles, medicines or personal care products and cosmetics or the authorisation of substances. The improved use of HBM data in health risk assessment (HRA) and in health impact assessment (HIA) for phthalates has been explored. So far, HBM data was used for the following evaluations under the REACH regulation: • Application for authorisation on formulation of recycled soft PVC containing DEHP in compounds and dry-blends • Application for Authorisation for DnBP used as an absorption solvent in a closed system in the manufacture of maleic anhydride • Restriction of DEHP, BBzP, DiBP, DnBP in toys and childcare articles (See D5.1)

Policy Question	Short Summary of Results
	In 2018 a case study on phthalates and bisphenols were conducted under WP 5.4 in which a structured and participatory process were developed to facilitate the use of HBM data and results by decision makers and also stakeholders. For phthalates specifically, it was mentioned that a major milestone achieved so far is the use of HBM data from the DEMOCOPHES project for health impact assessment, which severed the basis for the restriction proposal of 4 phthalates (DEHP, BBzP, DiNP and DnBP). This restriction would further restrict the use of these phthalates in consumer products in addition to the already restriction in place for childcare articles. It also made clear that substantiated data is needed to support policy making as former efforts to restrict or ban this substance group failed due to lack of data. Among the concerns were that the regulatory process still is very slow and only for some single substances progress has been made. In addition, the scientific methodology for assessing the risk of combined effect to this large group of chemicals is still under debate and too less individual data is made available to risk assessors in order to be able to make a detailed analysis to give a better insight in the actual cumulative exposure. It was also noted, that its regulatory policies are divided into domains which makes it rather difficult to prevent exposures if not all domains are implementing a restriction or ban. Representatives from the industry stated that EU companies are committed to innovation also because of stricter regulations. However, enforcement has been a bottleneck in the past. As a conclusion it can be said, that the follow-up of exposure trends of phthalates would help the EU agencies to evaluate whether the current regulations are effective enough or need to be adapted (e.g. are sources not adequately controlled by current regulation?). Also, it should be laid a focus on following up of how the substitution of the regulated phthalates develop in order to make prospective policy decisions. On that account, new methods for assessing the health risks to related substitutes but also for mixtures of phthalates and possibly other anti-androgenic substances should be developed. Furthermore, HBM4EU did participate in the open consultation processes of SCHEER on the “Preliminary guidelines on the benefit-risk assessment of the presence of phthalates in certain medical devices covering phthalates which are carcinogenic, mutagenic, toxic to reproduction (CMR) or have endocrine-disrupting (ED) properties” and of ECHA on the “Public consultation on behalf of the Commission: Update of Annex XIV entries of four phthalates” to feed in the expertise of the Consortium and ensure that results are directly fed into the regulatory processes.

21 Prioritised substance group: UV filters (benzophenones)

Policy Question	Short Summary of Results
Are sensitive reliable and cost-effective methods and biomarkers available to measure UV filters?	List of biomarkers, matrices and analytical methods is available (WP 9). UV-filters (benzophenones; BPs) are analysed as total, free or conjugated BPs. Determination of total or conjugated BPs include enzymatic hydrolysis. The extraction procedures consisted of dispersive liquid-liquid microextraction, SPE, and automated online SPE. The automated online SPE systems have the highest throughput. BP3 has been measurd in most laboratories. List of 8 candidate laboratories for analysis of benzophenones is available (WP 9.2). This list includes laboratories in Denmark, Germany, Norway, Sweden and Spain. Several expert laboratories were chosen to participate in Interlaboratory Comparison Investigations for analysis of BP-1 and BP-3. As part of WP8 (fieldwork preparation) AD7.2 included information on stability of BP-3 in urine. It was reported that urinary conjugates of benzophenone-3 (BP-3) are stable for one week when urine is stored at 4°C and for at least six months when stored at -70°C. In contrast, BP-3 conjugates commenced to degrade after three days when the urine was stored at room temperature.
What are current exposure levels to benzophenones in the EU population (cumulative exposure from different exposures sources)?	Harmonised aggregated data and exposure distributions for BP-1, BP-3 and the metabolite 2,2'-dihydroxy-4-methoxybenzophenone (DHMB) in urine, stratified by age and gender is available (WP 10-D10.6). These harmonised aggregated data are obtained from two data collections from Denmark: Democophes with exposure data for children and teenagers, and DYMS (Danish Young Men Study) with exposure data in teenagers; and one from Germany: Environmental Specimen Bank with exposure data in adults. Data is available from these collections on morning urine (Democophes), random spot urine (RegionH) or 24 hr urine (ESB). The heterogeneity of the data collections (different age range, different sampling types, different sampling years) makes it difficult to compare the levels between data collections. It appears that based on the Danish Democophes data collection that female children have slightly higher median urinary levels than male children for BP-3. Report on studies on UV filters in EU in adults, children, adolescents is available (WP7). Based on the 2018 questionnaire, there are 6 studies which included collection of data on UV filters, most of which are in the Northern region. No initiated or planned studied were reported on UV filters in children and adolescents, however one study was initiated in adults. There were more studies on children and adolescents compared to adults and elderly. Studies reported in the 2018 questionnaire collected data on UV filters in a range of biological samples including blood, saliva, urine, hair and umbilical cord blood. Planning of aligned studies is underway (WP8). The biomarkers included in the aligned studies are BP-1, BP-2, BP-3 and BP-7. Data will be collected in adolescents and adults in Sweden(?), Norway, Poland, Portugal and Spain. Evaluation of exposure levels in available literature on BP-3 as part of the risk assessment is ongoing (WP5.3)

Policy Question	Short Summary of Results
	Review on the available literature on Benzophenone-3 and -1 (WP13-WP14) includes map showing number of studies from EU countries  European countries for which publications reporting human benzophenone-3 (and -1) were identified. In the boxes the number of publications reporting BP-3 human exposure data is presented for each country. The number in the brackets represents the number of those publications, which provided information on BP-1 concentration as well. *HBM4EU WP13 working group on Benzophenones*
What are the major sources of exposure to benzophenones in the EU population and in vulnerable groups such as children and pregnant women?	Development of questionnaires for adults and adolescents was completed (WP7.3). The main variables in the questionnaire for UV filters cover use of cleaning products and scents in the home, consumption of food in food contact materials, use of cosmetics and hygiene products, type of sunscreen used, and DIY hobbies and activities. The statistical analysis of existing data (WP10 in collaboration with WP13-14) will be based on a meta-analysis and will include a section on main sources of exposure.

Policy Question	Short Summary of Results
Do exposure levels differ significantly between different EU countries (possibly related to climate)?	No., Available data from literature (studies between 2004 – 2017) on average urinary BP-3 levels from studies from Western Europe (4 studies), Southern Europe (1 study) and Northern Europe (19 studies) were compared in a random-effects meta-regression model. No studies with data on urinary BP-3 were identified from Eastern Europe. No significant difference in average urinary BP-3 levels between the three regions were observed when adjusting for sex, age and period of sample collection. (WP13)
Do exposure levels differ between different sub-groups: elderly, adults, and children? Between males and females? Between adults of different age groups? Between individuals in different ethnic subgroups (perhaps due to differences in use of sunscreen products)?	Exposure distributions BP-1, BP-3 and DHMB in urine, stratified by age and gender is available (WP 10, D10.6), however only 3 data collections were obtained. Reported average BP-3 levels in urine stratified by age and gender based on the literature is available for Northern Europe (WP13). No significant difference was seen between males and females in these European studies. Average urinary BP-3 levels were significantly lower in children and adolescence compared to adults.
Are current exposure levels safe in relation to the endocrine and carcinogenic properties of benzophenones? (for the general population and for vulnerable groups such as children and pregnant women)?	Appropriate effect biomarkers were identified (WP 14.1) These include: ○ Reproductive hormones: BP-3 could alter the androgen/estrogen balance based on experimental findings, and urinary exposure to BP-3 was associated with decreased serum TT levels in adolescent males in the NHANES. Thyroid hormones: Exposure to BP-3 is associated with altered thyroid hormone levels in several studies, including both pregnant women and adults. Experimental studies support that benzophenones may alter thyroid hormone balance by influencing their central regulation and metabolism and inhibition of thyroid peroxidase (TPO) appears as a potential mechanism. Moreover, effects may be more pronounced in a context of low iodide availability, still prevalent in many parts of the world. Risk assessment of BP-3 based on urinary levels and effect levels, taking into account the new evaluation of the SCCS is currently ongoing (WP 5.3) WP12 prepared a review of available PBTK models for benzophenones. Three human model PBTK models for benzophenones have been published.
Was the restriction of BP-3 in cosmetics in the EU (September 2017) effective in reducing public exposure? Did exposure to other benzophenone or other UV filter compounds increase as a result?	Data is not available to answer this question (no studies identified with sampling after 2017). There was no significant change in average BP-3 levels from 2003-2017 within the Northern European studies. Data from aligned studies may provide data to answer this question. However only BP-3 will be measured in these studies.