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2 Introduction

This deliverable reports on the achievements of HBM4EU in 2017 and 2018 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. AD5.2 presents what has been achieved so far per substance. The substance-specific reports will be also published on the HBM4EU web site under the substance-specific web pages.

3 Prioritised substance group: Phthalates & DINCH

Please note: Not all policy questions (PQs) are listed – only those which have been addressed until now with notably results. All 13 PQs can be found in the scoping documents.

Policy Question	Short Summary of Results
1. Which are the most sensitive, reliable and cost effective methods and biomarkers to measure phthalates and Hexamoll® DINCH®?	In Work Package (WP) 9 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 has 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.
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 statistical significant and toxicological relevant difference in mean concentration between adults and children? […]**	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 (reference values), time trends, geographic comparisons, and exposure determinants were defined. A requirement for the statistical analyses is the sharing of data as laid down in the Data Management Plan (DMP, see D5.1). Therefore, data need to be provided to the HBM4EU repository, a platform to share data on human subjects between HBM4EU partners which aims to reach the highest level of the General Data Protection Regulation (GDPR) compliancy. 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 (Newborns, 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

Policy Question	Short Summary of Results
between occupational exposed and non-exposed adults? […] between male and female?	reported were with children and these studies were mostly conducted in Western Europe. For studies representative for Europe, a deeper analysis of spatial and temporal data gaps will be done in the scope of WP10 and WP7. 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. Gaps in EU-representative data on exposure to phthalates and DINCH (e.g. missing regions and/ or exposure biomarkers) will be 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 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 in national studies, whereas Poland contribute with a regional study. For the Southern region, Slovenia, Greece and Italy will collect new samples in national and regional (Italy) studies. 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 a regional study (see D8.4).
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 (2014-2018). 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 below.

Policy Question	Short Summary of Results
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).
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). So far, Germany and Denmark have available and suitable data collections to be used in the time trend analysis, which will be finished in the second half of 2019. 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.
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?	In WP 5.3 an exercise were 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, RCR 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. 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

Policy Question	Short Summary of Results
	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.
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.

Policy Question	Short Summary of Results
	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 workshift. Currently, the derivation of HBM-GV is being finalised for the following substance: DPHP, DnBP. And the following HBM-GV are in consultation: DiBP and BBzP. Final consolidated values will be delivered in September and November 2019. In addition to consolidation with national expert, the EU Policy Board (particularly ECHA and EFSA) are included in the consultation phase. HBM-GVs 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. These values will together with the result of WP10 feed also into addressing the research question 8.
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. PhthalatesPhthalates 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 throughthrough 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

Policy Question	Short Summary of Results
	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).
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 identifyingidentifying 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 sourcessources, 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 followingfollowing 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) In 2018 a case study on phthalates and bisphenols were conducted underunder 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 phthalates in consumer products in additionaddition 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

Policy Question	Short Summary of Results
	exposure. It was also noted, that it regulatory policies are divided into domains which makes it rather difficult to prevent exposuresexposures 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 mixturesmixtures 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.

4 Prioritised substance group: Bisphenols

Policy Question	Short Summary of Results
1. What is the current exposure of the EU population to BPA?	***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 laboratorieslaboratories 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 will permit 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. At this stage, three rounds have been carried out and labs who succeeded will be available soon. 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.

Policy Question	Short Summary of Results
	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). 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. ***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. ***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 researchresearch questions on general exposure levels, time trends, geographic comparisonscomparisons and exposure determinants (see D10.2).
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 adults[1] 2) the BE value based on one hand on the pTDI from Health Canada and on the other the BE value based on the US EPA RfD and EFSA TDI[2]. 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 did exceed 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

[1] 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.

[2] 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.

Policy Question	Short Summary of Results
	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 will be released in 2019. ***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. 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 / neurological effects. The technical and scientific limitations have also been discussed (AD14.3). ***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 PBPTK 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). 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. 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. (Detailed results in D12.4)
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,

Policy Question	Short Summary of Results
	EHP, 2019)[3]. Using different combinations of these tools it was possible to identify the most likely toxic outcomes of exposure to these substituents. ***Putative toxic effects of BPA substituents***. Using the tools mentioned above it was possible to highlighthighlight 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).
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 and at the end of 2018 will take place the 2nd ICI round. 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, fueled by the discrepancy between standardized regulatory studies (used for formal risk assessments) that do not report health effects, and an increasing number of

[3] 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
	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.
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. BPA was proposed to be 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.
6. Are health risks age and gender dependent?	WP7 has run a NHCPs online consultation on existing HBM surveys. The outcome from 124 questionnairesquestionnaires 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 was among one of the most analysedanalysed substances.

Policy Question	Short Summary of Results
	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.
8. How can HBM feed into assessment of the Tolerable Daily Intake (TDI) for BPA, as set by the European Food Safety Authority (EFSA)?	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, 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 the enterohepatic recirculation modelling. 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 are working on the establishment of new HBM4EU health based guidance values for BPA both in the general population and in occupational settings. These values will be released in 2019.

Policy Question	Short Summary of Results
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.

5 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 contains the HBM4EU strategy with the overall aim to align ongoing and/or planned studies to collect data from HBM4EU priority substances with EU wide coverage. The current exposure will be assessed on the basis of samples between 2014 and 2019 related to three age groups. The strategy includes countries from different European regions focusing on children aged 6-11 years, adolescents aged 12-19 years and adults aged 20-39 years. In addition, an inventory on national HBM studies that could be part of the first HBM4EU Human Biomonitoring program was made. For PFAS, the proposed sampling scheme includes adolescents (12-19 years of age), and time trend analysis. Data will be collected in countries of all parts of Europe: Norway and Sweden (North), Slovakia (East), Slovenia, Spain, Greece (South) France, Belgium, Germany (West). Up to 12 PFAS will be measured in the respective samples: PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFBS, PFHxS, PFHpS, PFOS, sum of all isomers. These data will provide a basis for exposure and risk assessment for a part of the European population, namely teenagers. Within task 7.6, a post-harmonisation strategy for the collection of data (questionnaires) was required. For the collection of new samples and data WP7.3 basic questionnaires were developed which were further spitted into questionnaires for the single priority substances. With respect to PFAS, a basic questionnaire for adolescents aged 12-19 years was developed including additional specific single questionnaires for PFAS. In WP 8.4, influencing and interfering factors for sampling and storage were identified, whereas for each substance group recommendations were made to avoid sample contamination or inappropriate storage conditions that may influence sample quality and hence the outcome of the analysis. For PFAS, specific recommendations are given, e.g. avoiding Teflon and other fluropolymers as well as glass in the sampling material, and information on shipment and biobanking. In an additional deliverable (AD7.2), a literature research and a concept for a sample quality study on impact of thawing and freezing on integrity of human samples was conducted. PFDS, PFHxS, perfluorooctanoate and perfluorononanoate are stable in serum for at least 10 days stored at room temperature, and for at least 8 months stored at -20°C or below. Most PFSAs and PFCAs measured in water solution are stable for three months when stored at 4°C, but are adsorbed to the surface of PP containers rapidly, thus indicating the use of other sample containers like e.g. high-density PE containers even for short-term

Policy Question	Short Summary of Results
	storage. For budget estimates and to support the overall planning of the analytical work, tentative prices for biomarker analysis in HBM4EU were obtained within WP9.2. For PFAS, the range (min-max) of time spans required for the analysis of 300 samples were evaluated to be between 1 and 8 months depending on the specific laboratory responding to the request that have been made in this AD. The range of prices provided (by n=14 labs) for selected PFAS (and partially for additional PFAS) ranged between 70 and 641 € per sample (19,500 – 67,500 € for 300 samples). Overall, 21 labs were contacted to provide information, 14 of which responded. For the Quality Assurance/Quality Control Scheme in the HBM4EU project (ICI/EQUAS) (WP9.4), the first and second round of proficiency testing for the determination of PFAS in serum including PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFBS, PFHxS, PFHpS, PFOS (sum of all isomers) were concluded. The third round has started. Based on existing HBM data, D10.4 presents the first annual list of European reference values (ERV). First steps were made to calculate exposure distributions for existing HBM data on PFAS. Research protocols were developed. Currently, no harmonised aggregated data are available yet. As first results for PFAS, available HBM data from existing data collections from different time periods (without any limitations) and across different geographical European regions were collected. The majority of HBM data on PFAS is available from birth cohorts leading to a primary focus on this population group. 17 data collections were identified for PFAS analysis in maternal plasma/serum during pregnancy or in cord plasma/serum of the newborn. 14 of the respective data owners were invited to share their data for joint analyses. Data collections with too small sample (<120) were excluded. Until date of publication of D10.4 (12/2018), the owners of 10 data collections were willing to participate and 5 were interested in participating but needed approval from their authorities. Results are planned to be published in 2019. Within WP13.3 studies for the examination of exposure-health relationships were identified. For this, a dynamic inventory of existing studies was made. PFAS studies included in the inventory comprise 10 studies in pregnant women (all studies contain information on health outcomes), 18 studies in children and adolescents (out of which: 12 prospective cohort studies), and 13 studies in adults and the elderly (out of which: 5 prospective cohort studies). In the annual work plan 2018 is was outlined to merge individual data from several birth cohorts and to examine the associations between PFAS and low birth weight, and to examine associations with birth weight as continuous outcomes, especially for PFAS other than PFOS and PFOA. Thus, a detailed research protocol was written, and partners who agreed to share their individual data were identified. These data were collected. The results for PFAS are expected to be reported in M36. Additionally, it was decided to conduct similar analyse by merging individual data for PFAS to assess the associations between maternal PFAS concentrations and thyroid function in both mothers and their newborns. Several partners agreed to share their data. Reporting the preliminary results is anticipated in M36.

Policy Question	Short Summary of Results
	Within WP5.3 (D5.5), the use of HBM data in risk assessment (RA) and health impact assessment (HIA) strategies was demonstrated (further description of this work see answer concerning 11 PFAS mixtures). The recent EFSA opinion on PFOA and PFOS was used as starting point. According to EFSA the exposure of a considerable part of the European population exceedsexceeds the provisional tolerable weekly intakes also in case of using low exposure estimations. These exceedances could be also observed by comparison of internal human benchmark dose levels with levels detecteddetected in Human Biomonitoring studies from Europe. The Benchmark dose levels were based on increase of cholesterol, delayed response to vaccination in children and reduction in birth weight (EFSA, 2018).
2. Are there differences in exposure of the EU population to regulated and non-regulated PFASs?	As described above (policy question 1) efforts are underway to characterize the exposure of the EU population to PFAS, including the alignment of studies, the development of a post-harmonisation strategy, the planning of the analytical work, as well as the examination of exposure-health relationships and the inclusion of HBM data in risk assessment. Differences in exposure levels of the measured regulated and non-regulated PFAS will be assessed as well.
3. Has restriction of PFOS according to the POP Regulation led to a reduction in exposure, especially for children?	Within HBM4EU aligned studies on PFAS the selected age group are teenagers. Thus, no new data on exposure of children will become available. Within HBM4EU time trends could also be further explored within WP 10 using existing data, though limitations due to different study populations and geographical areas need to be considered. Within WP13.2 observations from birth cohorts could also bring further evidence. Concerning time trends observed in Human Biomonitoring studies the EFSA opinion on PFOS and PFOA in food states: Following an increase from the early 1970s, decreasing concentrations of PFOS and PFOA have been observed in many time trend studies after the year 2000 (EFSA, 2018).
4. Is exposure driven by diet, consumer exposure, occupation or environmental contamination?	Within WP12 food intake was found to be the most important contributive route to the exposure of PFOS and PFOA, with percentages of 97% and 98% of the total intake, respectively. These estimations were made based on a study from Catalonia (D12.1). Dietary exposure to PFOS and PFOA has been recently thoroughly assessed by EFSA. (EFSA 2018). 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.

Policy Question	Short Summary of Results
	Sampling is under way. The analysis of PFAS will be done if the ICI/EQUAS round for PFAS will be completed (WP9.4). The present study also includes the analyses of several effect biomarkers which are made mainly with the participants own funding. It is expected that the study will be completed by the end of 2019. In WP7.7, template materials to support the participation in HBM4EU surveys were developed. The template materials were transformed into tailored materials for each HBM4EU survey, which were translated into different languages. Specifically for PFAS, materials were developed related to the first aligned occupational HBM4EU survey (Exposure of European Workers to Hexavalent Chromium (Cr(VI)) and other chemicals), whereas targeted materials are available in English, German, Finnish, French, Italian, Dutch, Polish and Portuguese comprising the participating countries. Additionally, as stated in the answer to policy question 1, different work has been undertaken for the identification of the exposure of the EU population to PFAS, including the alignment of studies, the development of a post-harmonisation strategy, the planning of the analytical work, as well as the examination of exposure-health relationships and the inclusion of HBM data in risk assessment. As described above exposure of European teenagers will be investigated, this will be accompanied by the assessment of determinants of exposure.
5. Which areas and environmental media in Europe are contaminated with PFASs?	Within WP10 a substance-group-specific statistical analysis plan has been developed. Variables for assessing environmental contamination have been identified: place of birth, place of residence (near a fluorochemical industrial facility, near civilian airports, military bases, wastewater treatment facilities, or firefighting training facilities, near agricultural areas characterised by the use of soil conditioners),years of residence, consumption of tap water, use/consumption of groundwater or surface water, locally produced food, own grown vegetables, own raised livestock, fish and seafood from a local body of water. However, focus of HBM4EU is exploring the background exposure of the general population and no specific studies in known hotspot areas are planned. Though HBM4EU study materials can be used in national studies performed to investigate certain contamination cases.
6. How can this feed into an assessment of the TDI for PFOS and PFOA set by EFSA?	Suitable PFAS studies for the examination of exposure-health relationshipsrelationships were identified within WP13.3, including studies in pregnant women, children and adolescents, adults and the elderly. Additionally, it is planned to merge individual data from several birth cohorts for the examination of associations of PFAS exposure and low birth weight, and birth weight as continuous outcome. A similar investigation will be conducted for the assessment of associations between PFAS and thyroid

Policy Question	Short Summary of Results
	function in mothers and their newborns. Results are expected to be reported in M36. For more detailed information see answer to policy question No. 1.
7. What is the impact of a pending 2016 ECHA decision to restrict the manufacturing, marketing and use of PFOA under REACH?	New data which will become available within HBM4EU and can be compared with European data from earlier studies. According to EFSA, 2018 also a decrease in PFOA could be observed in Human Biomonitoring studies, including time trend analyses (EFSA, 2018)
8. Is it important to eliminate legacy PFASs from material cycles (i.e. waste electronic equipment) when implementing a circular economy in order to protect human health?	Due to the long half-life in humans, to exceedances of tolerable daily intakes and internal benchmark dose levels of substances which are already restricted such as PFOS and PFOA it seems indicated to eliminate PFAS from material cycles when implementing a circular economy in order to protect human health.
9. Can differences in PFASs profiles be observed in different population groups and time periods?	Efforts to assess PFAS exposure within HBM4EU are described above. Differences in PFAS profiles could be described by analysing time trend studies, which are not available at European level so far. European time trend studies will not be possible within HBM4EU, though they could be initiated.
10. What are the PFASs levels and health effects in vulnerable population groups?	As described above PFAS exposure will be examined in European teenagers. Within WP 14.2 (D14.3) biomarkers of effect according to their utility in human studies were selected. Though it is unclear which of them will and can be actuallyactually measured in the HBM4EU aligned study it was proposed to measure brain derived neutrophic factor, thyroid hormones and glucose markers, serum lipids and adipokines, beside neurobehavioural tests and antropometric tests. Suitable PFAS studies for the examination of exposure-health relationships were identified within WP13.2. (D13.3) including studies in pregnant women, children and adolescents, and adults and the elderly. Additionally, it is planned to merge individual data from several birth cohorts for the examination of associations of PFAS exposure and low birth weight, and birth weight as continuous outcome. A similar investigation will be conducted for the assessment of associations between PFAS and thyroid function in mothers and their newborns.

<table>
<tr><th>Policy Question</th><th>Short Summary of Results</th></tr>
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Results are expected to be reported in M36. For more detailed information see policy question 1. Further, it can be referredreferred to the EFSA assessmentassessment, compiling Human Biomonitoring and benchmark dose levels.

The table below depicts serum concentrations of PFOS and PFOA in European adult and children populations based on medians derived from studies reported in EFSA 2018 and the comparison with the derived BMDL5 levels by EFSA as reported in WP5.3 (D5.5.)
<table>
<tr><td>Age group</td><td colspan="2">Adults</td><td colspan="2">Children</td></tr>
<tr><td>Concentration</td><td colspan="4">ng/ml</td></tr>
<tr><td></td><td>PFOA</td><td>PFOS</td><td>PFOA</td><td>PFOS</td></tr>
<tr><td>BMDL5</td><td>9.2-9.4</td><td>21-25</td><td>9.2-9.4</td><td>10.5</td></tr>
<tr><td>Median*</td><td>1.9</td><td>7.7</td><td>3.3</td><td>3.2</td></tr>
<tr><td>Mean*</td><td>2.1</td><td>7.5</td><td>3.3</td><td>3.3</td></tr>
<tr><td>Minimum*</td><td>0.76</td><td>1.7</td><td>0.49</td><td>0.49</td></tr>
<tr><td>Maximum*</td><td>4.9</td><td>27.4</td><td>6.9</td><td>8.6</td></tr>
<tr><td>Number of studies</td><td>32</td><td>32</td><td>8</td><td>8</td></tr>
</table>

The critical effect (benchmark dose level benchmark dose for a 5% increase: BMDL5) identified in EFSA 2018 was the increase of serum cholesterol (9.2-9.4 ng/ml). Other benchmark dose levels established were delayed response to vaccination for children for PFOS: BMDL5: 10.5 ng/ml and effects on birth weight: PFOA: BMDL5: 4-10.6 ng/ml, PFOS: 21 ng/ml). Using individual data, for PFOS, the concentrations in adults and children ranged from 0.06 to 392 ng/mL and from 0.47 to 23 ng/mL, respectively.
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Policy Question	Short Summary of Results
	For PFOA, the concentrations in adults and children ranged from 0.03 to 81 ng/mL and from 0.45 to 19.5 (P95) ng/mL, respectively. Much higher concentrations of PFOS and PFOA are also the case for occupationally exposed adults & for persons experiencing elevated exposure from for instance contaminated drinking water.
11. How can mixture effects of environmental and human PFASs mixtures present to date be estimated?	Suitable PFAS studies for the examination of exposure-health relationships were identified within WP13.2 (D13.3.) including studies in pregnant women, children and adolescents, adults and the elderly. Additionally, it is planned to merge individual data from several birth cohorts for the examination of associations of PFAS exposure and low birth weight, and birth weight as continuous outcome. A similar investigation will be conducted for the assessment of associations between PFAS and thyroid function in mothers and their newborns. Results are expected to be reported in M36. For more detailed information see also Policy Question No. 1. First attempts to assess mixture effects of PFAS have been undertaken in WP 5.3. (D5.5). The challenges to date for assessing mixture effects of PFAS are the lack of endpoint specific toxicity data for PFAS preferable in humans as well as human exposure data. Currently EFSA is assessing the risk of PFAS mixtures, it is however not clear which PFAS will be included and which endpoint in which species will be selected. Within WP 5.3 (D5.5.) the use of HBM data in risk assessment (RA) and health impact assessment (HIA) strategies was demonstrated. The recent EFSA opinion on PFOA and PFOS was used as starting point. According to EFSA the exposure of a considerable part of the European population exceedsexceeds the provisional tolerable weekly intakes also in case of using low exposure estimations. Within work under WP5.3 (D5.5.)PFOA, PFOS, PFNA and PFHxS were considered for the RA in the general population, whereas cholesterol increase in humans for PFOS and PFOA, and hazard data based on animal data for PFNA and PFHxS, used to derive minimal risk levels by ATSDR, were used for the RA. When conducting the mixture RA for PFAS, great uncertainties were identified stemming from species differences with regard to toxicokinetics and toxicodynamics. This is specifically relevant for PFAS because of their unique properties. The mixture RA conducted based on animal data only indicated that there is no potential risk. However, the mixture RA conducted based on European HBM data using epidemiological data (cholesterol increase) for PFOS and PFOA and extrapolated hazard data using animal-based relative potency factors for PFNA and PFHxS indicated a potential risk of parts of the population to these substances. Based on this work, the following conclusions were drawn: (i) there is a need for human-relevant hazard and HBM data, in order to establish endpoint specific hazard indices with human relevance. For the majority of the 4,000 currently used PFAS considerable data gaps exist related to current uses, exposure patterns and toxicity. Especially, human-relevant exposure and hazard data is

Policy Question	Short Summary of Results
	needed for PFAS apart from PFOS and PFOA, (ii) there is a need for endpoint specific relative potency factors based on internal doses in humans, and (iii) there is a call for more intensive collaboration between toxicologists and epidemiologists to raise RAs to a higher level. In addition, several additional issues arose, such as how to handle vP substances within RA, the need for new methods and approaches for the grouping of chemicals and the prediction of their toxicity, and the validation of these methods. Within WP13.1 work on adverse outcome pathways is ongoing. Selected effects for contribution to the OECD AOP framework were based on the endpoints for which benchmark dose levels were derived by EFSA: effects on the liver accompanied by increase in cholesterol levels, effects on birth weight and effects on the immune system, all of those based on human data (D13.4). Certain AOPs in the AOP wiki database were identified which could be relevant for PFAS exposure in humans, however considerable data gaps related to causality and mode of action are lacking. Though, the mechanistic pathway from PFAS exposure to adverse health outcomes will be further explored. Within WP 14 biomarkers of exposure for PFAS were successfully established. Placental extracts (alpha fractions), containing mixtures of persistent and lipophilic chemicals, showed significant anti-androgenic activity. The hormonal profile from placental tissue was quantified, as well as some epigenetic markers such as Histone H2AX phosphorylation (Gamma-H2AX), trimethylation of histone 3 at lysine (H3K4me) and DNA methylation of BDNF, in addition to untargeted metabolomic analysis. Finally, 8OHdG levels were assessed in urine samples coupled to the placentas from the same women. This work has shown that chemical mixtures isolated from human samples can be assessed, and its biological activity quantified using different biomarkers cell- based tools. Placenta tissue could be used as a relevant biological matrix to assess both exposure and effect biomarkers. The placenta can also be used to explore the implementation of novel effect biomarkers in Human Biomonitoring programs, due to the volume and availability of this biological sample. Future work will focus on the relationship between exposure and effect biomarkers tested in placenta samples (included in D14.4 and AD14.4); ii) further to assess the implementation of the most appropriate biomarkers of effect and combined effects in other biological matrices more frequently recruited in HBM programs, such as blood and urine; to explore the concentration to specific chemical families, such as PFAS and metabolites, in placenta and serum samples, in order to assess its combined effects using different effect biomarkers such as some in vitro cell bioassays.

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?	As depicted above, for the majority of the 4,000 currently used PFAS considerable data gaps exist related to current uses, exposure patterns and toxicity. Especially, human-relevant exposure and hazard data is needed for PFAS apart from PFOS and PFOA, (ii) there is a need for endpoint specific relative potency factors based on internal doses in humans, and (iii) there is a call for more intensive collaboration between toxicologists and epidemiologists to raise RAs to a higher level. In addition, several additional issues arose, such as how to handle vP substances within RA, the need for new methods and approaches for the grouping of chemicals and the prediction of their toxicity, and the validation of these methods.
13. How can identification and assessment (including data on (potential) adverse effects on human health and the environment) of alternatives currently hampered by CBI (Confidential Business Information) be facilitated?	--
14. How much are HBM values dependent on host characteristics and does this have implications for identifying vulnerable groups?	A new PBPK model for PFOA and PFOS was developed based on a previously reported model within WP 12. (D12.1). For validation purposes, data on PFOA and PFOS in human tissues from people living in the area of study (Tarragona County) were used. The levels of 13 PFASs, including PFOA and PFOS, were reported in blood samples of 48 residents in that same area. In addition to the model validation, a study on the best partition coefficients was conducted. Hence, the model was tested by using, as input data, partition coefficients from studies conducted with either rats or humans. Data sets were compared to detect any improvement in the performance of both original and adapted PBPK models. Several discussion points have been identified, e.g. highlighting the importance to obtain partitioning data from humans and of PFAS levels in human tissues in order to refine the model. It could be further assessed if the model could be used to explore this question further.

6 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). Cohorts with available metadata were evaluated and it was determined that at this point spatial variations can be evaluated based on the available data. It is not possible to evaluate temporal trends with the currently available data, 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. There is insufficient available data to extend to the EU-wide population and/or other regions, and thus to directly evaluate the effect of the current legislative framework. To address the question of statistical analysis of spatial trends, a data analysis plan (task 10.4) has been created on “Geographic Variations in Category A Flame Retardants in the European Population”. This will incorporate records of serum and maternal milk from the four regions of Europe (Belgium, Denmark, France, Norway, Slovakia, Spain), and compare the geographic trends evaluated by the different matrices. This will serve as a baseline to eventually evaluate whether declines due to chemical regulation are uniform across the EU, once data from aligned studies can also be included in the statistical analysis.
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 will be produced for FR exposure in children age 6-11, for urinary biomarkers of organophosphate ester FRs, and serum biomarkers of BFRs by the end of 2019. 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.

Policy question	Results
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 modelling is crucial to enable comparison of the legacy and emerging FRs.
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 can be addressed through WP12, which includes exposure and PK modelling, where various hypotheses regarding differences in exposure pathways and distributions within the body can be addressed.
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 serum and maternal milk for 6 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.

Policy question	Results
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 will be used to aid in identification of relevant exposure pathways for FRs, beginning with BFRs.
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, but this has not yet been specifically applied to flame retardant mixtures. Under WP16, methods are being developed to screen for multiple FRs in single HBM samples, which can be used to provide information on the co-occurrence of FRs.
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.

Policy question	Results
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). It is anticipated that reference values for a subset of FRs will be possible with the inclusion of aligned data in 2020.

7 Prioritised substance group: Cadmium (Cd) and Hexavalent Chromium (Cr VI)

Policy Question	Short Summary of Results
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 harmonised 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.

Policy Question	Short Summary of Results
	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.
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 visualised 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.
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).
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).
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

Policy Question	Short Summary of Results
	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. 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, normalisation 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).
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

Policy Question	Short Summary of Results
	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.
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.
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.
What is the maximum acceptable level for Cd in food stuffs?	Work in progress within WP10 and WP12 (similarly as above)
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.

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Policy Question	Short Summary of Results
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).
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 were 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

Policy Question	Short Summary of Results
	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). 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 will be completed by the end of 2019.
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 characterise 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. In addition, WP10 has developed a substance-specific statistical analysis plan for priority substances including Cr(VI) (see D10.2). 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.
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). 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.
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

Policy Question	Short Summary of Results
	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 evaluate the exposure to Cr(VI) 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 exposure and health effects. This issue has been reported in D13.4 and D13.5 with a purpose to establish exposure-health relationships. WP13 did give a detailed overview of the available knowledge on AOPs for Cr(VI) (D13.4) 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 deliverable D13.5.
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 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/m^3 until 5 years after the transposition date and after that period the limit will be 0.005 mg/m^3. On the other hand, in France and the

Policy Question	Short Summary of Results
	Netherlands, an OEL of 1 µg/m^3 has been set for Cr(VI) in all uses. These are the most stringent OELs currently set in workplace in EU. Respect to these exposure levels available, we will able to answer this question after the analysis of samples of air and HBM samples of workers within the occupational chromate study.
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 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).
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 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) and industrial hygiene samples (air and wipes) were developed (as above reported). Moreover, SOPs were developed for any of these matrices (as above reported).

Policy Question	Short Summary of Results
	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 now completed. Additionally, 5 laboratories have set up the methodology for the analysis of Cr(VI) in EBC. Moreover, for EBC-Cr(VI) a small-scale interlaboratory comparison to ensure the quality of the analysis has also been performed. The first list of approved laboratories is now available (see D9.3) and includes 15 laboratories for Cr measurements.
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) by 5 countries 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.

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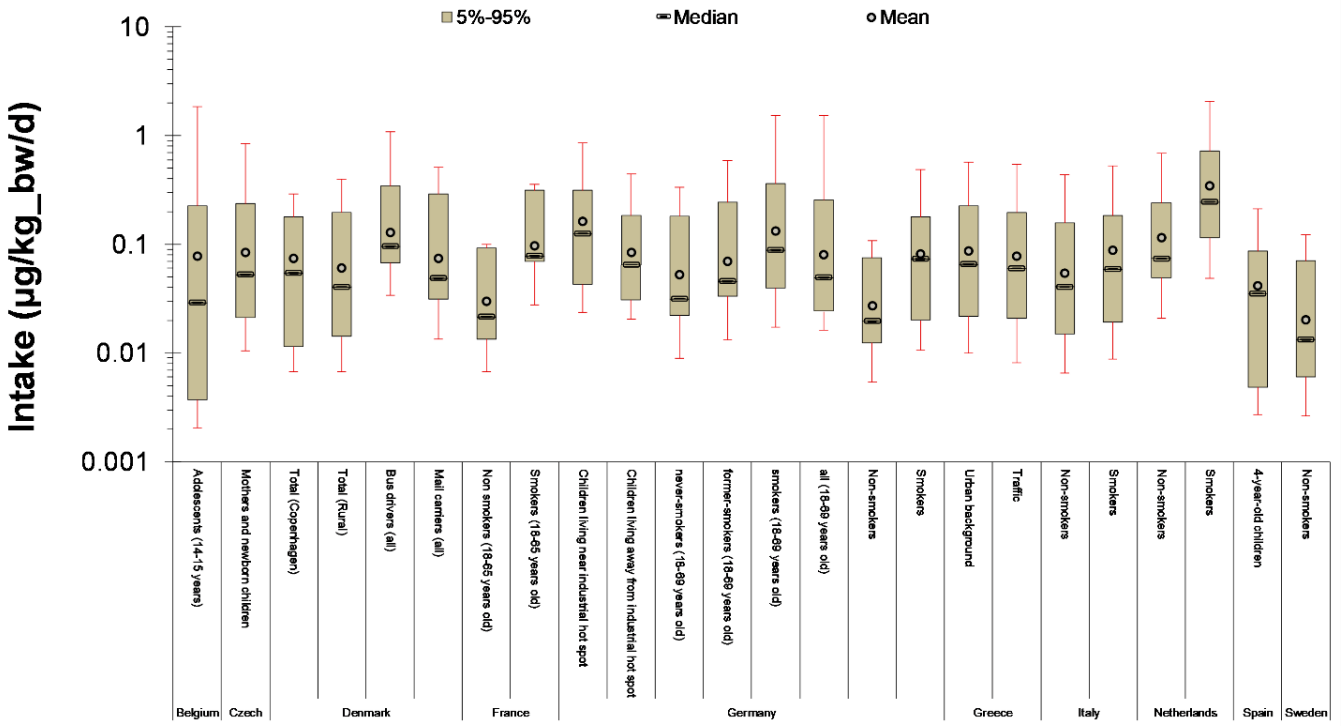
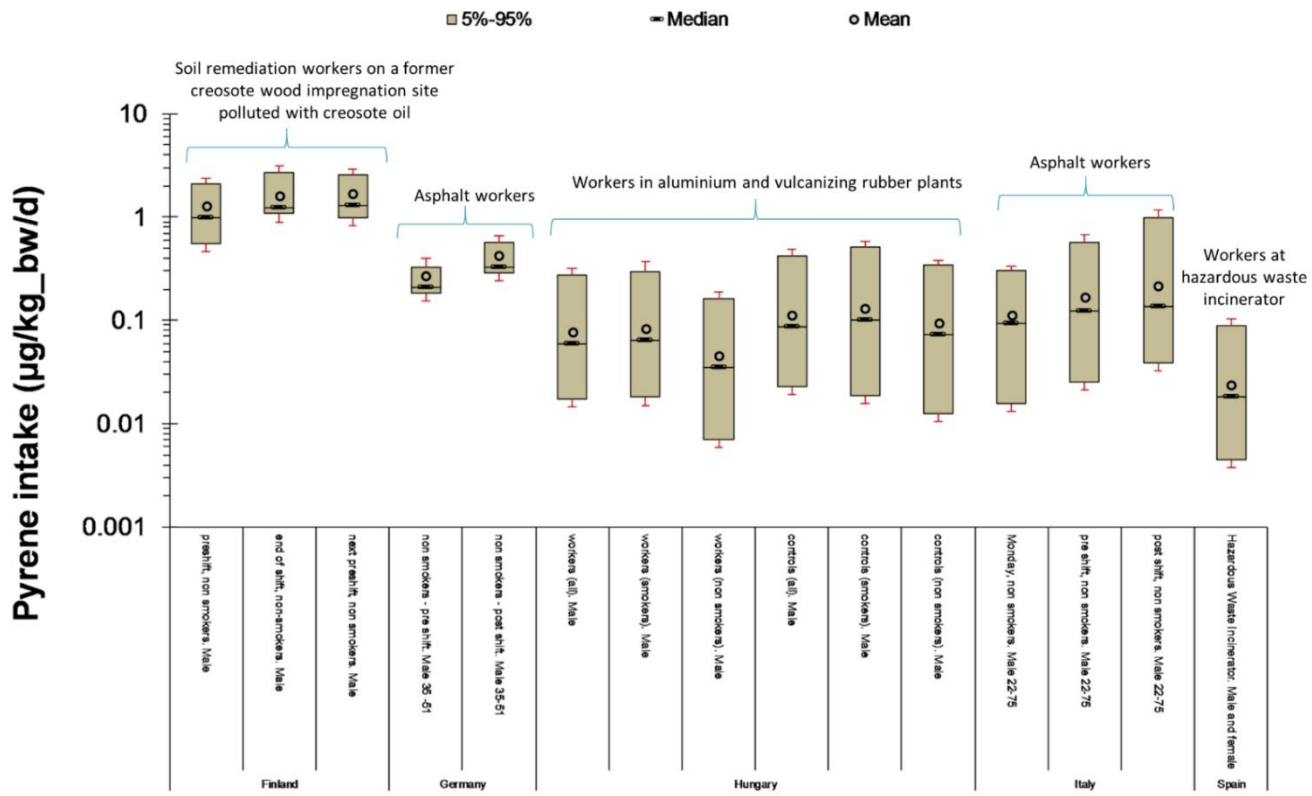
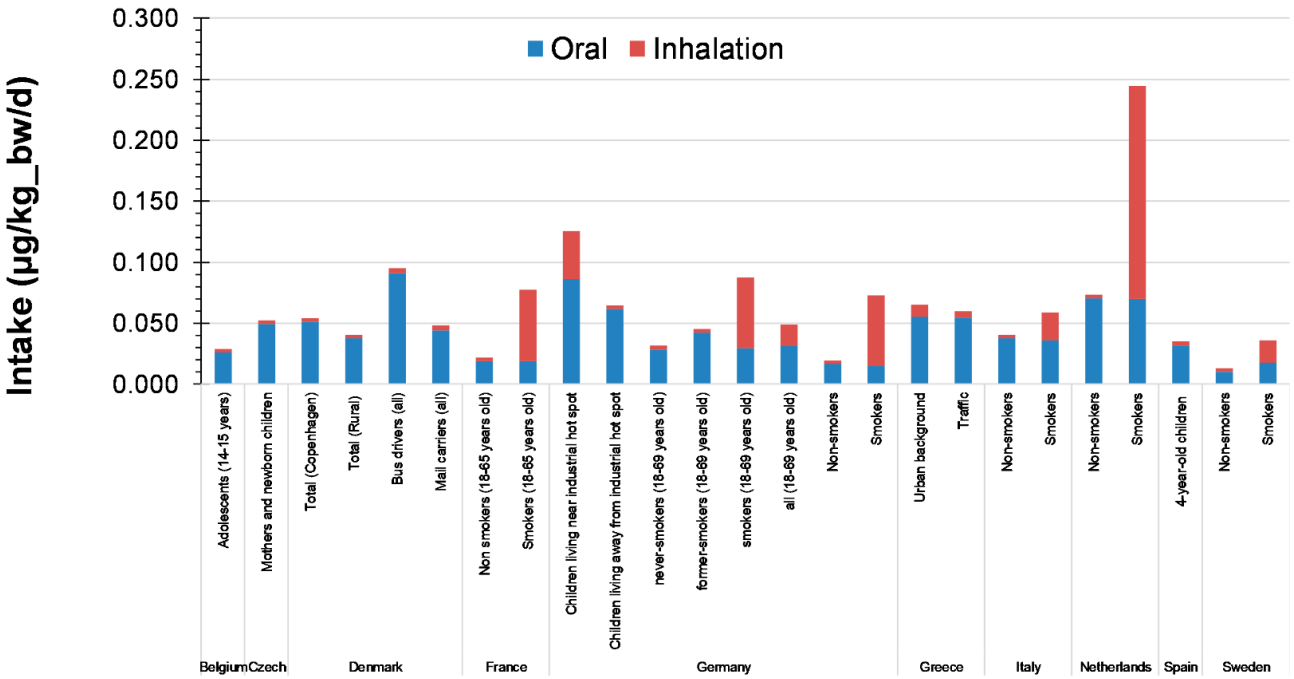
8 Prioritised substance group: PAHs and air pollutants

Policy Question	Short Summary of Results
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. The daily intake estimates for pyrene (regarding the general population) are illustrated in the following figure. 

Policy Question	Short Summary of Results
	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/kgbw/d, however, it has to be noted that, as described above, the bio samples were not collected in the same year, while analyses were performed by different laboratories, thus, hampering the overall intercomparison.
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 aluminium 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 aluminium 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 aluminium 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). A summary of the intake levels for occupational groups is illustrated below. In each occupational sector, a differentiation in the lifestyle of workers is exhibited based on smoking habits.

Policy Question	Short Summary of Results
	
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.

Policy Question	Short Summary of Results
Is there an association between air quality and human exposure to PAHs?	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 second-hand 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). 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. Exposure to air pollution has a marginal contribution to overall PAHs intake compared to diet; Diet is found to account for almost 90% of daily intake, while inhalation for less than 10%. However, in areas affected significantly by strong PAHs sources (such as proximity to refineries, industrially contaminated areas or waste incinerators), the contribution of air pollution exposure to PAHs is important and accounts for almost 20-30 % as illustrated in the figure below. It has to be noted that in the case of smokers, the red bar indicates the contribution from both smoking and inhalation of ambient air.

Policy Question	Short Summary of Results
	
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
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

9 Prioritised substance group: anilines

Policy Question	Short Summary of Results
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 diisocyanates 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 exists, the data is still limited. There are also some data on the occupational exposure to specific anilines (carcinogen o-toluidine and sensitizer 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 2019.
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.
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.

Policy Question	Short Summary of Results
	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. 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. The results of the modelling are comparable to those obtained earlier by using urinary mass balance based calculation approach. These results are used to calculate RCRs in D12.5. AOPs for anilines have been developed under WP13 to support human health risk assessment.
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 has started in June 2019. 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 diisocyanates and corresponding amine exposures is under preparation in 2019. This new study will bring us information to study the impact of the planned REACH restriction/EU OEL for diisocyanates.

10 Prioritised substance group: mixtures

Policy Question	Short Summary of Results
What is the information need of regulatory bodies and stakeholders?	The information needs on mixtures of regulatory bodies and to a lesser degree 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. We took the concept of 'information need' as developed by Robert S. Taylor in 1962 (Taylor 1962) as first point of departure. In Taylors view, information need has four levels: • The conscious and unconscious need for information not existing in the remembered experience of the inquirer. In terms of the query range, this level might be called the "ideal question" — the question which would bring from the ideal system exactly what the inquirer, if he could state his need. It is the actual, but unexpressed, need for information • The conscious mental description of an ill-defined question. In this level, the inquirer has a conscious information need in the mind and might talk to someone else in the field to get an answer. • A researcher forms a rational statement of his question. This statement is a rational and unambiguous description of the inquirer's doubts. • The question as presented to the information system. As witnessed from documents of the regulatory bodies and from the Horizon2020 Joint Mixture Project Cooperation discussions, the 'ideal question' remains implicit. For the governance of mixture risks, we therefore expressed the conscious mental description of the ill-defined question on information needs of mixtures as: "How can we effectively and efficiently manage the health risks associated with chemical mixtures in the European population in such a way that residual mixture risks are considered acceptable from a public health and personal health point of view, while maintaining to the degree possible the societal and personal benefits of the products that lead to the mixture exposures".

Policy Question	Short Summary of Results
	Effectively and efficiently would entail not only a 'utilitarian' perspective, but also an 'egalitarian' perspective, meaning that the optimal situation is not only at the population level, but also involves social justice elements, such that risk groups are also sufficiently protected. From this, we derived a number of 'rational statements' and the questions that are relevant to HBM4EU: 1. What would constitute an 'acceptable risk' for mixtures and how can this be operationalised? 2. What is the level of acceptable risk to society (regulators, experts, stakeholders, civil society) of the chemical manmade cocktail in the European population? 3. Is this acceptable risk level exceeded, given the mixtures observed from HBM data in the European population? 4. What are the main drivers and sources of unacceptable mixtures risk level? 5. What are the action perspectives to reduce an unacceptable mixtures risk level? 6. Which actors are involved in the risk reduction and how? 7. What is the optimal mix of actions to reduce the risk effectively and efficiently? 8. Does the current regulatory system support the implementation of such sets of optimal risk reduction actions? In the structure of Taylor, a set of questions were derived for semi-structured interviews. 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.

Policy Question	Short Summary of Results
	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. 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 were 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 are for the first time, being applied to real HBM mixture data (FLEHS) under bilateral agreement. Results are expected in the second half of 2019. Other existing data are being lined up for subsequent analysis and for across country comparisons.
Can we identify hotspots or risk groups with high mixture exposures?	In the absence of (access to) existing HBM mixture data (see above) this questions could not yet be addressed. In parallel to the analysis of existing HBM mixture data, a joint survey on pesticides was developed. The first field work for this survey will start in the fall of 2019. Urine samples will be collected in 50 parent-child pairs in hotspots (residences within 500 m of agricultural application of pesticides) and 50 parent-child pairs in control areas. Samples will be collected in two consecutive days in a non-spraying and a spraying season. Samples will be analysed through pesticide suspect screening in conjunction to CGL Emerging Chemicals (WP16). Details of the joint pesticide survey are described in AD15.7.

Policy Question	Short Summary of Results
Which sources & pathways contribute most to HBM mixture values?	In the absence of (access to) existing HBM mixture data (see above) this question could not yet be addressed.
Which effect markers can we use to assess health risks of mixtures?	So far, this question remains largely unanswered. WP14 has made suggestions for mixture effect biomarkers in conjunction to the pesticide study, i.e. 8OHdG and 8-isoprostane biomarkers in urine. Therefore, these biomarkers are included as an add-on in the joint survey on pesticides. Also, in response to questions from the mid-term review, further opportunities to include effect biomarkers in the joint survey on pesticides will be explored. However, since only urine samples are included, options are limited. The Swiss partners in the joint survey on pesticides only study adults in a larger population sample; they may be able to expand the effect indicators. In four of the five case studies on Task 15.2 'Identification of mixture health effects', in cooperation with WP13 and WP14, suggestions for effect biomarkers are expected as additional results of the case study. Moreover, activities on priority chemicals of specific chemical families may provide effect biomarkers of the combined group of chemicals in that family. However, the 'mixture' perspective explicitly goes beyond single chemical families.
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 (see D15.1 for full list). These were mainly drawn and paraphrased from actual discourse on mixtures. These include: • Excessive exposures to mixtures of chemicals are best managed by personal (lifestyle) choices • Excessive exposures to mixtures of chemicals are best managed by regulation within my DG (e.g. Sante, Environment, Employment, Growth or their national equivalents) • Excessive exposures to mixtures of chemicals can only be managed by regulation jointly across different DGs • To reduce excessive exposure to mixtures, the biggest contributors to these risks should be regulated first and their contributions reduced

Policy Question	Short Summary of Results
	• To reduce excessive exposure to mixtures, all contributors to these risks should be regulated simultaneously and their contributions proportionally reduced • To reduce excessive exposure to mixtures, the contributors that are cheapest to regulate and reduce should be tackled first • To reduce excessive exposure to mixtures, the contributions from products/applications with the least favourable benefit/risk ratio should be regulated and reduced first • To reduce excessive exposure to mixtures, involuntary exposures should regulated first, since citizens have no influence on them • To reduce excessive exposure to mixtures, reduction of voluntary exposures by citizens is the most effective strategy • To reduce excessive exposure to mixtures, intentional mixtures should be first targeted • To reduce excessive exposure to mixtures, mixtures originating from a single source should be first targeted • Technological improvements and innovations will automatically reduce exposure and body burdens to mixtures of chemicals • Current legislation is adequate to reduce excessive exposures to mixtures through targeting intentional mixtures • Current legislation is adequate to reduce excessive exposures to mixtures through targeting unintentional mixtures from a single source • Current legislation is adequate to reduce excessive exposures to mixtures through targeting coincidental mixtures from multiple sources and through multiple pathways • Mixture risk assessment and management needs to be explicitly addressed in relevant regulations prior to any effective mixture risk management to take place • Mixture risk assessment is sufficiently developed to start implementation in risk management, even though it is not yet explicitly addressed in regulations • Expert committees should explicitly address relevant mixture information, even where this is not required by regulations • Mixture risk assessment methodology is currently not sufficiently mature to be incorporated in the regulatory context • Application of standard safety/assessment factors are sufficiently conservative to incorporate potential mixture effects • Additional safety/assessment factors are needed to incorporate potential mixture effects • Policy makers are responsible to reduce the exposure to mixtures, as industry can only be held responsible for the safety of their products, regardless of any mixture contribution

Policy Question	Short Summary of Results
	• If safer substitutes are available, irrespective of the risks, chemicals should be replaced by these safer substitutes to reduce mixture risks • Unintentional and coincidental mixtures are impossible to regulate, because each person is exposed to different mixtures every day 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 is needed to gauge support for these (sometimes incompatible) alternative action perspectives to reduce mixture risks in the population. Such an activity is currently not planned and should be developed in conjunction with Pillar 1.

11 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. Several of the “emerging” substances will be measured for the first time in recently collected (>2014) biobanked urine or blood samples of the general European population. The strategy is described in D8.4 (WP8). HBM4EU focuses on substitutes and alternatives for hazardous substances such as the phthalate substitute DINCH that will be analysed in samples from children (NO,DK, HU,SK,SL,PL,EL,IT,NL,FR,DE) and teenagers (NO,SE,PO,CZ,SK,SL,EL,ES,FR,BE,DE). The organophosphorus flame retardants that replace the brominated flame retardants will be measured in samples from children (NO,DK,HU,SK,SL,PL,EL,IT,NL,FR,DE). Some of the newer perfluorinated compounds will be analysed in samples from teenagers (NO,SE,PO,CZ,SK,SL,EL,ES,FR,BE,DE). The bisphenols BPS and PBF that substitute BPA, will be analysed in adult samples (DK,IC,FI,PL,CZ,HR,PT,FR,CH,DE,LU). WP9 has selected the most suitable biomarkers for the first set of prioritised substances (D9.2) and has built qualified laboratory capacity for targeted screening (D9.3). To identify new emerging substances, WP16 began with applying the suspect screening capabilities already existing in several EU laboratories/countries to analyse human urine and blood samples from various cohorts, leading to new detection frequencies data for a range of chemicals (D16.3). WP16 is then preparing a specific library aiming to expand and better harmonise these existing capabilities (D16.4). A first 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 (D15.2

Policy Question	Short Summary of Results
	and AD16.2). A second proof-of-concept is related to the non-targeted identification of halogenated markers of exposure and toxicological concern in human samples (D16.3). This work is developed from various biological matrices, with a special emphasis on 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. The same approach is planned to be 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. D16.1 "Prioritised list of known emerging chemicals" has listed existing international databases of emerging chemicals as a useful tool to orientate the selection of compounds to be characterised. The list contains more than 70 000 entries of pre-identified emerging chemicals and metabolites with unique structural and stereochemistry properties and their exact masses. The curated data serve as a reference for HRMS screening. AD16.2 has developed a framework for identifying which emerging chemicals should be prioritised. The probability of presence in human samples combined with the probability of toxicity (e.g. based on computational tools such as QSARs or effect directed analysis) will be used to rank compounds for inclusion in biomonitoring programs.

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 inventoried 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 (D16.1) and a first workflow for screening emerging chemicals (D16.2) has been published on the HBM4EU web site. **A chemically driven approach**: D16.2 highlights 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. 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). As proof of concept this has been applied on 25 placenta samples that 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).