

Scoping documents for 2021 for the first and second second round HBM4EU priority substances

Deliverable Report

D4.9

WP4 - Prioritisation and input to the Annual Work Plan

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2 Abstract / Summary

This deliverable contains updated scoping documents for the first and second round substances that were prioritised in HBM4EU. For each substance group, scoping documents are produced under Work Package 4, Task 4.4 of HBM4EU. The scoping documents contain a review of the available evidence, list policy-related questions, identify knowledge gaps and propose research activities.

Proposed activities are fed into the framework of work packages and tasks of HBM4EU in a coordinated and harmonised manner, and constitute the basis for the annual work plans. The scoping documents are the linkage between policy questions and the research to be undertaken (broken down for single substances) in order to answer those questions. This methodology will optimise work on the different substances, avoid redundancies, ensure coordination and facilitate the calculation of budgets for each WP.

The scoping documents do not contain a comprehensive literature review per substance group but are intended to provide information for the WP leaders who will draft the Annual Work Plans.

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3 Categorisation of substances

The aim of HBM4EU is first to get information on the human internal exposure of (potentially) toxic substances and substance groups in the EU population(s), and secondly, to be able to interpret the internal exposure (biomonitoring) information in terms of health consequences in relation to sources and pathways of (aggregated) exposure. To enhance transparency for selection of chemical substances to be included in HBM4EU research activities, substances are categorised. Criteria for categorisation of substances in HBM4EU are mainly based on the availability of Human Biomonitoring data for each substance, its regulatory status, hazard information and the availability of analytical methods for biomarker analysis. The aim is to be fully transparent about knowledge gaps that might be addressed through Human Biomonitoring activities under HBM4EU.

Substances will pass from Category E over D, C, B towards Category A as more information becomes available. Fully characterised substances should end up as category A substances. Activities related to the categories B to E substances which are integrated in the HBM4EU work plans should serve to increase the level of knowledge on these substances and move them into a higher category, ideally into the Category A.

The allocation of substances to the categories A to D is based on an expert judgement using the information in the background documents. Category E substances should directly be addressed under WP16 dedicated to the emerging substances.

The categorisation supports the prioritisation process and indicates the information gaps hence allowing developing targeted activities in HBM4EU to fill the knowledge gaps. Categorisation provides a first idea on the total knowledge for each substance from the perspective of Human Biomonitoring, related to possible health consequences.

The categories A to E are described here below:

- ▶ **Category A** substances are substances for which HBM data are sufficient to provide an overall picture of exposure levels across Europe, and interpretation of biomonitoring results in terms of health risks is possible. Risk management measures have been implemented at national or European level. Improvement of knowledge for these substances will therefore focus on policy-related research questions and evaluation of the effectiveness of existing regulatory measures.
- ▶ **Category B** substances are substances for which HBM data exists, but not sufficiently to have a clear picture across Europe. Also, knowledge on the extend of exposure, levels and impact on the human health should be improved, in order to give policy makers relevant and strategic data to establish appropriate regulations and improve chemical risk management. Analytical method and capacities to monitor the substances across Europe might have to be improved.
- ▶ **Category C** substances are substances for which HBM data scarcely or doesn't exists. Efforts to develop an analytical method to obtain relevant HBM results need to be done. Hazardous properties of the substances are identified, yet greater knowledge on toxicological characteristics and effects on the human health is needed. Interpretation of HBM data is not possible, due to the lack of HBM guidance values.
- ▶ **Category D** substances are substances for which a toxicological concern exists but HBM data are not available. HBM4EU research may be focused on the development of suspect screening approaches permitting to generate a first level of data enabling to document the reality of human exposure and better justify further investment in a full quantitative and validated method development.

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- ▶ **Category E** substances are substances not yet identified as of toxicological concern and for which no HBM data are available. A bottom-up strategy will be applied, consisting to non-targeted screening approaches coupled to identification of unknowns capabilities for revealing, and further identifying, new (i.e. not yet known) markers of exposure related to chemicals of concern for HBM (parent compound or metabolite).

Substance groups are expected to include a range of substances, distributed across categories.

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4 Introduction

HBM4EU has established a strategy for deriving prioritised substance groups that HBM4EU will work on in 2019 and 2020. This stepwise strategy included input from national and EU policy makers and from stakeholders. The substances were nominated and prioritised according to a transparent procedure that is described in Deliverable 4.3 on the Prioritisation strategy and criteria, produced by ANSES. The detailed description of how this prioritisation strategy was implemented in practice, the inputs received and the methodology applied for selecting substances to include in the second list of prioritised substances is the subject of the Deliverable D4.4 (lead EEA).

First, a survey was launched to understand the demands of the National Hubs, EU policy makers and members of the HBM4EU Stakeholder Forum. Subsequently an online survey requested the nomination of substances for research under HBM4EU. A long list of new nominated single substances and substance groups was produced. Substances on the long list were ranked according to the number of nominations received, enabling to reduce the list down to a short list of approximately 25 substances. Background documents on the substances on the short list were produced. An expert group of HBM4EU scientists scored and ranked the substances according to their hazardous properties, exposure characteristics; and public concern. The ranked list was discussed at a joint meeting of the HBM4EU Management Board and the European Union (EU) Policy Board in March 2018, where agreement was reached on the draft 2nd list of HBM4EU priority substances. The Governing Board approved the final list. The Governing Board members were asked to identify a list of candidate institutions and experts for the positions of Chemical Substance Group Leaders for the new substances/groups of substances. The substance group leaders were approved and were asked to produce the scoping documents for the new list of prioritised substances. The process is documented in D4.5 Second list of HBM4EU priority substances and Chemical Substance Group Leaders for 2019-2021. First scoping documents for the 1st set of prioritised substances as of June 2017 is documented in D4.2 and for the 2nd set of prioritised substances as of November 2018 in D4.6 and its update in D4.7.

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5 Prioritised substance group: Acrylamide

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5.1 Background information

5.1.1 Hazardous properties

Acrylamide (also named 2-propenamide, acrylic amide, ethylene carboxamide, structural formula: $\text{CH}_2=\text{CH}-\text{CO}-\text{NH}_2$) is a low molecular weight, highly water soluble, organic compound produced for different uses in chemical industry. The concern about hazardous exposure arose in 2002 when acrylamide was discovered to be formed in certain high carbohydrate food at high temperature. From experimental animal studies, acrylamide has been shown to have neurotoxic, carcinogenic, genotoxic and mutagenic effects (category 1B, CLP classification) and also possible/suspected immunotoxic and developmental toxic (category 2 CLP classification) effects as well as adverse effects on the reproductive function in particular in males (1-4). In humans, occupational exposure to acrylamide has been shown to cause neurotoxicity in the peripheral nervous system through prolonged or repeated exposure. Other toxic effects of acrylamide in humans are still under investigation. Although epidemiological studies have not consistently observed an increasing risk of common cancers in relation to dietary acrylamide, there is a concern about its possible carcinogenic effects in humans (IARC classification 2A: probably carcinogenic to humans; SVHC: substance of very high concern). Glycidamide, its main metabolite, is considered to represent the main metabolite, from which the genotoxicity and carcinogenicity of acrylamide originate. A recent study found glycidamide mutational signature in a full one-third of approximately 1600 tumor genomes corresponding to 19 human tumor types from 14 different organs (5). Evidence from a limited number of epidemiological studies suggests that acrylamide may also negatively affect fetal growth (5, 6). It may also cause allergic reactions if in contact with the skin (7). There is no consistent evidence in humans that acrylamide may act as endocrine disruptor. A possible adverse effect of mixtures of acrylamide and other chemical compounds, particularly other carcinogens in food should be taken in consideration for the risk assessment and needs to be further investigated (8, 9).

5.1.2 Exposure characteristics

Acrylamide is manufactured and/or imported in the European Economic Area in 100 000 - 1 000 000 tonnes per year. According to REACH registration data, the substance was readily biodegradable in a screening test and is, therefore, not considered to be persistent. The substance does not bioaccumulate since it has a very low octanol-water partition coefficient. Release to the environment of acrylamide may occur from the industrial use: manufacturing of the substance, as an intermediate step in further manufacturing of another substance (use of intermediates) and for thermoplastic manufacture. Acrylamide is most commonly found in water and soil but rarely found in air. However, it is expected to be highly mobile in both water and soil. When released to land, acrylamide does not bind to soil, and move rapidly through the soil column and into ground water. It is removed from soil through enzyme-catalysed hydrolysis and it does not bioconcentrate in aquatic organisms (10).

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5.1.2.1 Human related exposure sources and uses

The industrial and laboratory use of acrylamide mainly concerns the production of polyacrylamides, which are used primarily as flocculants, mainly for clarifying drinking water and treating wastewater. Acrylamide and polyacrylamides are also used in the production of dyes, organic chemicals, permanent-press fabrics, textiles, pulp and paper products. In the oil industry, acrylamide is used as a flow control agent to enhance oil production from wells. Beyond the chemical industry use, acrylamide is used in building and construction (e.g. as grouting agent and soil stabiliser for the construction of tunnels, sewers, wells, and reservoirs), health service, and scientific research (10). Moreover, in 2002 it was observed to be generated during food processing at temperatures above 120 degrees Celsius under low moisture conditions. It is formed predominantly by food containing asparagine and reducing sugars via Maillard reaction when processed at high temperature such as frying, roasting and baking (not boiling). The main food sources of acrylamide are coffee (and solid coffee substitute), potatoes fried products (including potatoes and vegetables crisps), biscuits, cereals and other products such as roasted nuts, olives in brine, prunes and dates and baby food. Protein-based foods (such as meats) probably contain low amounts of acrylamide (11). Acrylamide is also present in tobacco smoke.

5.1.2.2 Human exposure routes

Humans are exposed through inhalation, ingestion and the dermal uptake.

Oral uptake through the ingestion of food, cigarette smoke and water is the predominant exposure route for the general population. For occupational exposure, inhalation and dermal contact at the workplace where acrylamide is used or produced is another important route of acrylamide exposure. Moreover, transplacental exposure should also be taken in consideration for the risk assessment, although more investigation is needed (6, 12, 13).

5.1.2.3 Human Biomonitoring (HBM) data availability

Acrylamide exposure is assumed to be widespread among the general population. The most vulnerable groups for the possible adverse effect of acrylamide exposure are infants, toddlers, children and pregnant women. Of note, workers at the industrial site and manufacturing have also been shown to be highly exposed (14). Several epidemiological studies have been performed to investigate the association between occupational exposure to acrylamide and dietary acrylamide, and risk of cancer, neurological alterations and fetal growth (11). However, the exposure was mainly self-estimated (e.g. questionnaire based job history and dietary intake). Human Biomonitoring data on acrylamide are few and not been measured or published, respectively, in population representative studies up to now, in particular in Europe (5, 6, 15-20). Biomarkers of acrylamide have been identified (see Technical aspect section for details). The use of these specific and sufficiently sensitive biomarkers would represent a reliable indicator of dose instead of estimations based on self-reported data.

5.1.2.4 Health based guidance values available for HBM data

Since acrylamide and its metabolite glycidamide have been shown to generate genotoxicity and to be carcinogenic at any level, a tolerable daily intake (TDI) cannot be defined. Instead, health guidance values can be expressed as margin of exposure (MOE) and Benchmark Dose Lower Confidence Limit (BMDL₁₀). From animal studies, BMDL₁₀ values of 0.43 mg/kg body weight (b.w.) per day have been selected for peripheral neuropathy and of 0.17 mg/kg b.w. per day for neoplastic effects.

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The current levels of dietary exposure to acrylamide seem not to be of concern with respect to non-neoplastic effects (MOE > 10000 or higher) The mean daily exposure of adults in Europe is estimated to be between 0.4 and 0.9 µg acrylamide/kg b.w. (EFSA, 2015). Calculating the MOE for peripheral neuropathy results in MOEs between 478 and 1075. That is well below 10,000. It is highly questionable, whether the MOE concept should be used for the risk assessment of non-neoplastic effects. However, although the epidemiological association studies have not demonstrated evidence for acrylamide being carcinogenic, the MOE values indicate a concern for neoplastic effects based on animal evidence (11).

Biomonitoring equivalents (BE) - estimates of the concentrations of acrylamide and its metabolites in blood and urine - have been proposed as screening tools for interpreting HBM data for acrylamide in relation to non-cancer and cancer related effects (21). The non-cancer reference dose for acrylamide, established by the United States Environmental Protective Agency (USEPA), corresponds to a BE value for mercapturic acid of acrylamide (AAMA), a urinary biomarker of acrylamide, of 16 µg/g creatinine. The USEPA reference doses for cancer, based on an additional lifetime cancer risk of 1×10^{-4} and 1×10^{-6} , correspond to 2 µg AAMA/g creatinine and 0.02 µg AAMA/g creatinine, respectively (19). For other BE values for acrylamide and for the description of how these values were estimated, please refer to Hays et al. (21).

For occupational exposure to acrylamide, the derived no- or minimum effect level (DN(M)EL), level of exposure above which a human should not be exposed, is also available: inhalation exposure, long term DMEL 70 mg/m³ and short term (acute) 120 mg/m³; dermal exposure, long term DMEL 100 µg/kg b.w./day and short term DNEL 3 mg/kg b.w./day.

5.1.3 Policy relevance

Regulatory measures have been taken at the EU level. Acrylamide is registered under REACH and included in the candidate list of substances of very high concern (SVHC) due to its possible carcinogenic and mutagenic effect. Based on the inclusion in the registration list Annex XVII of REACH, after 5 November 2012 acrylamide should not be placed on the market or used as a substance or constituent of mixture in a concentration equal or greater than 0.1% by weight for grouting applications. Acrylamide has also a harmonised classification under the Classification Labelling & Packaging (CLP) Regulation. The European Drinking Water Directive 98/83/EC has set a parametric value of acrylamide in water for human consumption of 0.10 µg/L. The parametric value for acrylamide refers to the residual monomer concentration in the water as calculated according to specifications of the maximum release from the corresponding polymer in contact with the water. Acrylamide is also listed in the Annex II as substance prohibited in cosmetic products. Precautions for this substance have been recommended by industries under REACH. Moreover, since 2007 acrylamide levels in food are monitored according to a EU Recommendation (further extended Commission Recommendation 2013/647/EU and 2010/307/EU). Use of acrylamide is banned in plastic material and articles intended to come in contact with food (Commission Regulation (EU) No 10/2011 of 14 January 2011). Recently, the EU established mitigation measures and benchmark levels for reducing levels of acrylamide in food (Commission Regulation (EU) 2017/2158).

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5.1.4 Technical aspects

Acrylamide is extensively metabolised, mostly by conjugation with glutathione but also by epoxidation to glycidamide. Both acrylamide and glycidamide might be measured in serum. Serum concentration of acrylamide and glycidamide have both been shown to be highly correlated to acrylamide exposure but they have a short half-life. Other biomarkers include the urinary metabolites of acrylamide, mercapturic acids of acrylamide and glycidamide (AAMA and GAMA, respectively), and the hemoglobin adducts of acrylamide and glycidamide (AAVal and GAVal). Urinary metabolites are stable compounds and can be quantified with high specificity and sensitivity. They are measures of metabolic deactivation of AA and GA but are not directly related to critical target tissue doses. The hemoglobin adducts have a lifetime of about 110 days and have been shown to have high correlation with acrylamide exposure (21). Analytical methods for the determination of the aforementioned biomarkers are available and are characterised by the use of liquid- or gaschromatography (HPLC or GC, respectively) with detection by tandem mass spectrometry (MS/MS) using multiple reaction monitoring (MRM).

5.1.5 Societal concern

The societal concern is mainly related to the discovery that acrylamide is produced in processed foods rich in carbohydrates making acrylamide a widespread exposure. From the public perspective, different actions have been taking by several organisations. Chemsec, an independent organisation aiming to solicit legislators to speed-up in the decision-making process, has included acrylamide in the Sin List (Substitute it Now!) of the chemical compounds that can cause cancer, alter DNA or damage the reproductive system (CMR, class I&II). Acrylamide has also been included in the Trade Union Priority List with priority number 3, score 43. Non-governmental organisations (Safe Food Advocacy Law, and Changing Market and SumOfUs) call for mandatory EU limits of acrylamide in food after the “public scandal” related to the finding that acrylamide levels were clearly above the new benchmark level set by the European legislation in 2017, according to results from analyses of samples of potatoe crisps available on the market; in seven out of eighteen samples the acrylamide level exceeded the benchmark level.

5.2 Categorisation of Substances

Table 5-1: Substances included in the substance group, listed according to availability of toxicology and human biomarker data, in category A, B, C, D, E substances (see general introduction)

Category	Abbreviation/ Acronym	Systematic name	CAS No.	Regulation
B	AA	Acrylamide	79-06-1	REACH Regulation Annex XVII (Restriction List) REACH SVHC (candidate list) as carcinogenic (57a) and mutagenic (57b) CLP Regulation as carcinogenic, mutagenic (1B) and reprotoxic (2) IARC classifications 2A, probably carcinogenic to humans

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5.3 Policy-related questions

1. What is the current exposure of the EU population to acrylamide?
2. Are the exposure levels a concern for health? Is the exposure to acrylamide associated to cancer, neurological alterations and fetal growth in humans? Is the health risk dependent on long term or intermittent exposure to low quantity of acrylamide?
3. Does the exposure to acrylamide differ significantly between countries and population groups? Are the main reasons for these differences related to different dietary habits or to other factors?
4. Are the health risks dependent on age and gender?
5. Which population groups are more at risk? Are there other sources of exposure of acrylamide that need to be discovered (e.g. smoking habits or other food sources)?
6. Is there a possible mixture of effect between acrylamide and other carcinogens (particularly dietary carcinogens e.g. benzopyrene) ?
7. Is there an impact from the mitigation for the production in food processing and manufacturing and REACH restrictions on the distribution of acrylamide exposure? Do we need to implement other restrictions to decrease the level of exposure of acrylamide?

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5.4 Research Activities to be undertaken

Table 5-2: Listing of research activities to be carried out to answer the policy questions

Policy related questions	Substance	Available knowledge	Knowledge gaps and activities needed
1) What is the current exposure of the EU population to acrylamide? (Policy makers)	Acrylamide	<p>Malmö and Diet Cancer Cohort, Sweden blood n=142 adults (1991-1996)</p> <p>EPIC, FR, SE, DE, UK, IT, GR, NL, ES, DK, blood n=510 adults (1992-2000)</p> <p>Danish Cancer and Health cohort, blood n=377 post-menopausal women (1993-1997)</p> <p>Moba, Norway, blood, n= 79 women mother pregnant (1999–2008)</p> <p>CAPS, Sweden, blood n=377 adult men (2001-2002)</p> <p>Urban et al., Germany, blood, urine, n= 60 adults (2002)</p> <p>Fuhr et al., urine n=6, 16, 5 ; ?, men and women, men (no time measurements)</p> <p>Kutting et al, Germany blood, n=1033 all age and sex (2002-2004)</p> <p>Schettgen et al, Germany, blood n=104 (no time measurement)</p> <p>Boettcher et al., Germany, blood, n= 29 (no time measurement)</p> <p>Kellert et al., Germany, urine, n = 38 adults (no time measurement)</p> <p>Chevolleau et al., France, blood, n = 68 adults (no time measurement)</p> <p>Bjellaas et al., Norway, blood, n= 50 adults (no time measurement)</p> <p>NewGeneris, GR, ES, NO, UK, DK, blood, n= 1,101 mother/child (2006-2010)</p> <p>Heudorf et al., Germany, urine, n= 110 children (no time of measurement)</p>	<p>Lack of HBM data of the general population for most of the EU countries.</p> <p>Actions:</p> <ul style="list-style-type: none"> ▶ to generate new data based on samples from the aligned studies. Target group: occupationally exposed and general population. Based on the high content of acrylamide in certain foods (for instance baby foods and potato chips) new data should be generated in all age groups: ▶ new born (0-6 months) ▶ children and adolescents ▶ adults (middle ages and elderly, men and women) ▶ derive EU-HBM-HBGVs for workers and for the general population based on the Biomonitoring Equivalents for non-cancer Reference Dose (RfD). (Policy makers) <p>Relevant WPs: WP7, WP8, WP10</p>

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Policy related questions	Substance	Available knowledge	Knowledge gaps and activities needed
		<p>Kotova et al., Sweden, blood, n= 58 adults (no time of measurement)</p> <p>Mojska et al., Poland, urine, n= 78 women (2012)</p> <p>Carlsson et al.,Sweden, blood, n=51 children (2014)</p> <p>Goerke et al., Germany, urine, n= 20 adults (2015)</p> <p>GerS V, Germany, urine, n=1,450 children (2014-2017)</p> <p>Bioval/Bettermilk, Spain,urine, n=666 children, 120 pregnant women (2015-2022)</p>	
<p>2) Are the exposure level a concern for health? Is the exposure to acrylamide associated to cancer, neurological alterations and fetal growth in humans? Is the health risk dependent on long term or intermittent exposure to low quantity of acrylamide? (Additional questions)</p> <p>3) Does the exposure to acrylamide differ significantly between countries and population groups? Are the main reasons for these differences related to different dietary habits or to other factors? (Additional questions)</p> <p>4) Are the health risks dependent on age and gender? (Additional question)</p> <p>5) Which population groups are more at risk? Are there other sources of exposure of acrylamide that needs to be discovered (e.g. smoking habits or other food sources)? (Additional and policy maker questions)</p>	Acrylamide	<p>Evidence from animal studies have pointed out that acrylamide may be carcinogenic, mutagenic genotoxic, neurotoxic and have adverse effect on fetal growth.</p> <p>See also HBM studies listed above.</p>	<p>Findings from human studies are inconsistent and Human Biomonitoring is limited in Europe (5, 6, 15-20). Risk assessment is needed for both occupational settings and general population.</p> <p>Actions:</p> <ul style="list-style-type: none"> ▶ generate new HBM data for EU populations where there is a gap that can be considered in further HBM programs.(Policy makers) ▶ include acrylamide in general population surveys at national level to assess the EU population's exposure to acrylamide. (Policy makers) ▶ create occupational survey to assess whether workers are protected by acrylamide exposure. ▶ estimate the risk of certain endpoints (fetal growth, neurological alterations and cancer) in relation to acrylamide exposure from results of current and new epidemiological studies.(Additional) ▶ data analysis to identify current exposures levels, temporal and geographical trends and data gaps. (Additional)

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Policy related questions	Substance	Available knowledge	Knowledge gaps and activities needed
			<ul style="list-style-type: none"> collection, comparison and evaluation of existing data and integration into IPCheM.(Policy makers) <p>Relevant WP: WP7, WP8, WP10, WP11, WP13</p>
6) Is there a possible mixture effect of acrylamide and other carcinogens? (Additional question)	Acrylamide		<p>There is limited knowledge on a mixture effect of acrylamide and other carcinogens, particularly dietary carcinogens</p> <p>Actions:</p> <ul style="list-style-type: none"> To perform investigations for better understanding of mixture effects of acrylamide and dietary carcinogens e.g benzopyrene. (Additional) <p>Relevant WP: WP15</p>
7) Is there an impact from the mitigation for the production in food processing and manufacturing and REACH restrictions on the distribution of acrylamide exposure? Do we need to implement other restrictions to decrease the level of exposure of acrylamide? (Additional questions)	Acrylamide	Restrictions, monitoring, mitigations and prohibitions have been implemented for acrylamide in chemical industry, cosmetic products and in food. This might have decreased the exposure to acrylamide. A recent EU regulation aiming to reduce the level of acrylamide in food does not seem to have been respected by the food industries.	<p>There is lack of evidence regarding how the level of exposure of acrylamide has been affected after the adaptation of EU regulations aimed to decrease the level of exposure.</p> <p>Action:</p> <ul style="list-style-type: none"> To evaluate whether the EU regulations had an impact on the reduction of exposure level of acrylamide and whether other restrictions should be implemented for the food industry. (Additional) <p>Relevant WP: WP10</p>

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6 Prioritised substance group: Anilines

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6.1 Background Information

Aniline is the simplest member of the primary aromatic amines, in which one or more hydrogen atoms of the benzene ring are replaced by amino (-NH₂) group. Derivatives of aniline include a wide variety of different substances. Some of these (like benzidine and MOCA) are composed of two combined aromatic rings.

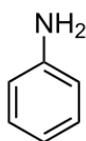


Figure 1: Structure of aniline, the simplest member of the aniline group

Many aromatic amines may cause methemoglobinemia in humans. Aniline and many of its derivatives are known or suspected human carcinogens. Several aniline derivatives can also cause skin sensitisation. Classical members of this family are bladder carcinogens 2-naphtylamine and benzidine, which use has been restricted in EU and there is therefore no exposure to these compounds. Aniline compounds are also formed as degradation products from e.g. azo-colourants, pharmaceuticals and from aromatic isocyanates used for polyurethane polymers, lacquers, foams and adhesives. Search from European Chemicals Agency (ECHA) website from authorisation list, candidate list of substances of very high concern (SVHC) and registration lists with a search term “aniline” results in more than 2000 search results. Several aniline derivatives can be found also from the list of substances restricted under REACH.

When looking at the aniline substances which are produced or imported in EU areas according to ECHA registration database at amounts above 1000 tonnes per year (tpa) and which have significant health hazards (other than only irritation/corrosion) the following substances can be retrieved:

- ▶ aniline, CAS: 62-53-3, harmonised classification in EU; H301, H311, H318, H317, H331, H341, H351, H372, H400
- ▶ o-toluidine, CAS: 95-53-4, harmonised classification in EU; H301, H319, H331, H350, H400
- ▶ 4,4'-methylenedianiline (4,4'-MDA), CAS: 101-77-9, harmonised classification in EU: H317, H341, H350, H370, H373, H411
- ▶ 4,4'-methylenebis[2-chloroaniline] (MOCA), CAS: 101-14-4, Harmonised classification in EU: H302, H350, H400, H410
- ▶ p-toluidine, CAS: 106-49-0, harmonised classification in EU: H301, H311, H319, H317, H331, H351, H400
- ▶ 1,3-diphenylguanidine, CAS: 102-06-7, harmonised classification in EU; H302, H315, H319, H335, H411, H361f
- ▶ p-phenylenediamine, CAS: 106-50-3, harmonised classification in EU; H301, H311, H319, H317, H331, H400, H410

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Many anilines have been registered for intermediate use only. These include for example 4-aminoazobenzene, 4-methyl-m-phenylenediamine, 6-methoxy-m-toluidine, 5-nitro-o-toluidine, 4,4'-methylenedi-o-toluidine. Although also these compounds have serious health hazards, they are not considered further because of the limited exposure due to intermediate use. Below, some anilines are discussed in some detail.

6.1.1 MOCA, MDA and diisocyanates

4,4-methylenebis(2-chloroaniline) (MOCA) and 4,4-methylenedianiline (MDA) are currently authorised under REACH. Both of these chemicals are genotoxic carcinogens to which a threshold for carcinogenic effects cannot be assigned. Both MOCA and MDA are easily absorbed via the skin. Therefore, biomonitoring is the best method for assessing occupational exposure to them. MDA is also a degradation product and a metabolite of MDI, one of the diisocyanates.

MOCA

MOCA is mainly used as a curing agent of the polyurethane products. It has a low vapour pressure and it is well absorbed through the skin. Therefore biomonitoring is the best method to assess occupational exposure to it. Exposure to MOCA can be biomonitoring by measuring MOCA excreted into the urine (free and conjugated MOCA). These methods are well established and used in occupational surveillance of workers. ECHA has recently made a dose-response analysis for the carcinogenicity of MOCA and calculated cancer risk levels for different urinary MOCA levels measured as total urinary MOCA in the end of the work-shift in the end of the work week (ECHA, 2015a). Also the EU Scientific Committee on occupational exposure limits (SCOEL) has recommended a biological guidance value (BGV) for MOCA (SCOEL, 2013). There is one application for authorisation for MOCA (ECHA, 2016a). It covers up to 89 sites in EU using MOCA as a curing agent in polyurethane production. Estimated number of exposed workers in EU is, however, only about 200. Authorisation has been applied for 12 years. There is, however, no European Commission (EC) decision nor ECHA's Risk Assessment Committee (RAC) and Socio-Economic Analysis Committee (SEAC) recommendation on the authorisation available yet.

The applicant has used biomonitoring data to assess the workers' exposure to MOCA. In addition, there are established methods available and published studies, especially from UK, on the biomonitoring of MOCA. Since there are substitutes for MOCA available for the use in polyurethane production, the use of MOCA may cease within becoming years when companies are able to move to the substitutes.

Therefore, MOCA might not be a very relevant candidate for further studies under HBM4EU although biomonitoring of MOCA would still be needed in EU as long as there are authorised uses in the EU. Furthermore, biomonitoring in workers should reveal a decrease over time (monitoring policy effectiveness). The general population is not exposed to MOCA, and the levels of MOCA and its metabolites in the urine of the general population are below the detection limits.

4,4'-MDA

Similarly to MOCA, the production and use of 4,4'-MDA is authorised under REACH. Like MOCA, also 4,4'-MDA is well absorbed through the skin and biomonitoring is the best method to assess occupational exposure to it. There are well established methods for the biomonitoring of 4,4'-MDA, which are based on the analysis of total urinary MDA excretion. The risk assessment committee (RAC) of ECHA has derived a dose-response for the carcinogenicity of MDA and calculated cancer risk levels for different urinary 4,4'-MDA levels measured as total urinary 4,4'-MDA in the end of the work-shift in the end of the work week (ECHA, 2015b). There are only two applications for authorisation under REACH. They concern 1) the industrial use of an epoxy resin hardener

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containing technical MDA aimed at immobilising spent ion exchange resins in a high containment matrix and 2) the formulation of MDA mixtures for this use. For these uses, there are RAC and SEAC opinions available and a 12 years review period has been proposed for these uses (ECHA, 2017ab). Total number of exposed workers in these uses is 56. The applicant of the authorisation provided biomonitoring datasets on the exposure of workers in these uses, and these data were used by RAC in the assessment of excess cancer risk to workers. Due to the limited use (other than intermediate use) and limited number of workers exposed to MDA, occupational exposure to 4,4'-MDA in its industrial use is not a good candidate for further work under HBM4EU.

Diisocyanates

MDA is one of the degradation products and main metabolites of methylene diphenyldiisocyanate (MDI, CAS 101-68-8). Measurement of urinary MDA can be also used to measure the occupational exposure to MDI. Similarly, toluene diamine (2,4-TDA or 2,6-TDA) can be used as a marker for exposure to toluene diisocyanate (TDI, CAS 584-84-9 for 2,4-TDI and 91-08-7 for 2,6-TDI). These diisocyanates are widely used in different applications (e.g. foams, sealants, coatings) throughout the EU, total volume in commerce is 2.5 million tpa (ECHA, 2016b). These diisocyanates (together with non-aromatic hexamethylene diisocyanate, HDI) are important occupational respiratory sensitisers; they are causing several thousand new cases of respiratory allergies (mainly asthma) annually in Europe. 4,4'-MDA (and isomers) is also the major cause of non-compliance of black nylon kitchen utensils imported from China, and the continuous EC testing requirement under the food contact materials legislation EC 10/2011. The source is likely from recycled polyamide (nylon), and from polyamide containing isocyanate lacquers used to coat the glass fibre reinforcement in the utensils. Aromatic isocyanates are also used in adhesives for laminated flexible plastic food packaging. (Mortensen et al. 2005, Trier et al. 2011). Aromatic Polyurethane polymers are also used in medicinal utensils, e.g. for stomi-bags, as nets operated into patients, in blood bags and tubings, as breast implants from where metabolites have been released and measured in the patients' blood causing sensitisation.

The use of the diisocyanates MDI, TDI and HDI has been recently proposed to be restricted in EU unless specific conditions for workers training and risk management measures apply. The aim of the restriction is not, however, to ban the use of diisocyanates but rather to improve the control of diisocyanate use by obligatory training for good working practices and risk management. In addition, there is work going on related to the setting of an OEL for diisocyanates under EU Chemicals Agents Directive (98/24/EC). Diisocyanate sensitisation can occur at very low exposure levels, and sensitive methods to assess exposure e.g. by measurement of diamine levels in urine are still needed in the future.

There is a need to study the possibility to improve the specificity and sensitivity of the current diisocyanate monitoring methods, and the effectiveness of the possible restriction on the occupational exposure to diisocyanates. Especially exposure to diisocyanates at small and medium sized enterprises is a concern.

There is also a need to better understand the exposure routes of isocyanates, e.g. via air, direct skin contact, or via ingestion of aerosols in order to target risk management measures correctly. In addition, sensitive biomonitoring methods, together with air and skin monitoring methods, are needed for the assessment of the effectiveness of the personal protective equipment. More about diisocyanates see specific scoping document on diisocyanates.

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6.1.2 Aniline and paracetamol

Aniline has been assessed under the existing chemicals regulation in EU (ESR, the pre-REACH EU-wide chemicals legislation). It is currently classified as a suspected carcinogen (carc cat 2) under the Classification, Labelling and Packaging Regulation (CLP) in the EU. In addition to the concerns related to the genotoxicity and carcinogenicity, it can cause methemoglobinemia and haemolytic anaemia after long term exposure. Major use of aniline is as an intermediate in the production of different chemicals, including rubber chemicals, dyes, some pesticides, drugs and polyurethane based polymers. It is also used in pH regulators and water treatment products and may also be formed in the thermal degradation of MDI-based polyurethane and reactions in rubber industry. Smoking is also a source of exposure to aniline. The EU risk assessment report from 2008 (based on the ESR) concludes that there is a need to limit the risk especially for workers but also for the general population near the point sources and consumers due to residues in different products. The main cause of concern is its carcinogenicity and genotoxicity (<http://echa.europa.eu/documents/10162/d537626b-e5b6-43e9-a7d2-582468edcc24>). Toxicity of aniline has been recently assessed also by SCOEL. There are validated biomonitoring methods available for aniline, and e.g. SCOEL has recommended a biological limit value based on the measurement of p-aminophenol in urine (SCOEL, 2016). It is also possible to measure aniline itself from the urine or haemoglobin adducts from blood samples. There are some biomonitoring data available both of the general population and workers exposed to aniline. Aniline has not been currently listed as SVHC substance, nor is it subject of any restrictions under REACH.

However, it is listed in the PACT-RMOA list under REACH, which includes substances for which a risk management option analysis (RMOA) or an informal hazard assessment for PBT/vPvB (persistent, bioaccumulative and toxic/very persistent and very bioaccumulative) properties or endocrine disruptor properties is either under development or has been completed since the implementation of the SVHC Roadmap commenced in February 2013. RMOA for aniline was completed in December 2015 and concluded that no action is needed at this time. However, it was noted that the recent exposure data was limited for both workers and for consumers (RIVM, 2015). Further regulatory actions on the aniline could benefit of additional data on both occupational and general population exposure to aniline. A metabolite of aniline, N-acetyl-4-aminophenol, is a commonly used drug, paracetamol, which can cause severe liver toxicity if used at high amounts. Ubiquitous exposure to paracetamol among general population have been demonstrated by Holger Koch's group (Modick et al 2014) who also detected measurable paracetamol levels in the Danish Democophes samples from 2011 (Nielsen et al 2015). The studies from Denmark related self-reported paracetamol intake of the mothers and her reporting of child intake to the biomonitoring of paracetamol among general population, including children and found no clear associations indicating an unknown source (Jensen et al.2014, Nielsen et al 2015, Graungård et al 2016).

o-Toluidine is classified as carcinogenic, cat 1B (May cause cancer; H350). It is manufactured and/or imported in the European Economic Area in 10 000 - 100 000 tpa. SCOEL has recently published a recommendation on o-toluidine, which includes also a recommendation for a biological guidance value (SCOEL, 2016). Although there are published methods for the biomonitoring of o-toluidine, limited biomonitoring data is available. The main uses of o-toluidine include its use as a curing agent in epoxy resins and an intermediate in producing azo dyes and pigments, acid-fast dyestuffs, triarylmethane dyes, sulphur dyes, indigo compounds, photographic dyes and synthetic rubber and rubber vulcanising chemicals. The largest use is, however, as an intermediate in the manufacture of herbicides. Earlier it was used in dyes and pigments. o-Toluidine is banned from cosmetics by the EU Cosmetics Regulation, also the use of azo dyes that release o-toluidine during degradation is not permitted for textiles and other consumer articles in the EU. Still, there are recent reports describing hairdressers exposure to it via the hair waving products (Johansson

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et al., 2015). Cherry et al (2011) has estimated that the number of o-toluidine exposed workers in EU is about 5500, mainly in the manufacture of other chemicals.

Taking into account that exposure may still occur via hair waiving products, the actual number may be higher. Also general population is exposed to background levels of o-toluidine.

p-Toluidine (4-aminotoluene) is manufactured and/or imported in the European Economic Area (1 000 - 10 000 tpa). It is classified as suspected carcinogen (H351). Its main use is in the manufacturing of other chemicals, including dyes, pigments, lubricants and polymer additives. Smoking causes exposure to p-toluidine and it is found in urine in the general population. In hairdressers, no increased exposure to p-toluidine compared to the exposure of general population was seen in a single study (Johansson et al., 2015).

p-PDA

p-Phenylenediamine (CAS 106-50-3) is a common contact allergen present in cosmetics and e.g. in hair dyes and e.g. tattoo inks. It has caused many occupational allergies e.g. among hairdressers exposed due to the contact with hair dyes. It has also been found in black nylon kitchen utensils, like 4,4'-MDA. It has not been regularly biomonitoring, although analytical methods for the analysis of it or its metabolites in urine or blood have been published. In these studies exposure of hairdressers to p-PPD has been described. The main hazardous property of p-PDA is its skin sensitising ability. It has not been listed as SVHC substance, nor is it subject of any restrictions under REACH. However, it has been listed in the PACT-RMOA list under REACH, which includes substances for which a risk management option analysis (RMOA) or an informal hazard assessment for PBT/vPvB (persistent, bioaccumulative and toxic/very persistent and very bioaccumulative) properties or endocrine disruptor properties is either under development or has been completed since the implementation of the SVHC Roadmap commenced in February 2013.

In addition, some of the available studies describe potential exposure to other sensitising aromatic diamines, like 2,5-TDA, m- and p-aminophenols due to the hair dyes. For example, EU Scientific Committee on Cosmetic Products (SCCP, 2007) has concluded that 2,5-TDA is very potent sensitizer and its use in hair dyes cannot be considered safe based on the available data.

Other high production volume (HPV) aniline compounds

Other substances manufactured/imported in EU >1000 tpa include 1,3-diphenylguanidine (CAS 102-06-7). No biomonitoring studies were found. It is manufactured and/or imported in the European Economic Area in 1 000 - 10 000 tpa.

1,3-diphenylguanidine is used in polymers and manufacturing of rubber and can be released in the environment from many construction, textile, furniture and rubber materials. Few occupational contact allergies have been reported due to 1,3-diphenylguanidine. It is classified as suspected of damaging fertility (H361). It has been subject for substance evaluation under REACH and there are some concerns on its potential genotoxic activity. Another comment raised during the evaluation process relates to the degradation products which may be formed e.g. during rubber manufacturing. These may include e.g. aniline.

Anilines manufactured or imported (in commerce) in EU at amounts of 100-1000 tpa include following substances:

- ▶ N,N-diethylaniline (CAS 91-66-7), in commerce in the European Economic Area (EEA) in 100 - 1 000 tpa and finds its main uses in the manufacture of other chemicals and in textile treatment products and dyes, rubber and polymers. It is classified as toxic via all routes of exposure and causing organ damage in long term exposure.

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- ▶ N-1-naphthylaniline (CAS 90-30-2); which is manufactured and/or imported in the EEA in 100 - 1 000 tpa and finds its main uses in lubricants and greases, polymers, metal working fluids and hydraulic fluids as well as in the manufacture of rubber products. It is harmful when swallowed and classified as causing damage to organs through prolonged or repeated exposure. It may also cause skin sensitisation.
- ▶ N-ethyl-N-[2-[1-(2-methylpropoxy)ethoxy]ethyl]-4-(phenylazo)aniline (CAS 34432-92-3) which is manufactured and/or imported in the EEA in 100 - 1 000 tpa and finds its uses in polishes and waxes, lubricants and greases, adhesives and sealants, washing & cleaning products, fillers, putties, plasters, modelling clay, inks and toners, leather treatment products, paper chemicals and dyes, polymers and textile treatment products and dyes. It is classified as harmful if swallowed, may cause damage to organs through prolonged or repeated exposure, and may cause skin sensitisation.
- ▶ p-(2,3-epoxypropoxy)-N,N-bis(2,3-epoxypropyl)aniline, (CAS 5026-74-4) and m-(2,3-epoxypropoxy)-N,N-bis(2,3-epoxypropyl)aniline, (CAS 71604-74-5) which are manufactured and/or imported in the EEA in 100 - 1 000 tpa per substance. The para-isomer is used in the manufacturing of other substances. There is limited information on the uses of the m-isomer. Both isomers are classified as suspected of causing genetic defects, it may also cause organ damage in long term exposure and skin sensitisation.
- ▶ 1,1'-(p-tolylimino)dipropyl-2-ol (CAS 38668-48-3) which is manufactured and/or imported in the EEA in 100 - 1 000 tpa and finds its main uses adhesives and sealants, coating products, fillers, putties, plasters, modelling clay, non-metal-surface treatment products and polymers. It is classified as fatal if swallowed.
- ▶ dapsone or diaminodiphenyl sulfone (CAS: 80-08-0) which is manufactured and/or imported in the EEA in 100 - 1 000 tpa and finds its main uses in polymers, adhesives and sealants as well as manufacturing of other chemicals, plastics, and rubber. It is also a widely used antibiotic for leprosis and some other diseases. It is classified as harmful.
- ▶ 4,4-oxodianiline (CAS 101-80-4) is an aromatic amine, which is on the candidate list of SVHCs due to its carcinogenic and mutagenic properties. It is manufactured or imported in the EEA in 10-100 tpa and used in the production of polymers.

For these, no systematic data search have been performed but according to the available information only limited/no biomonitoring data exists for these compounds.

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6.2 Categorisation of Substances

Table 6-1: Substances included in the substance group, listed according to availability of toxicology and human biomarker data, in category A, B, C,D,E substances (see general introduction)

Cat.	Abbrev./ Acronym	Systematic name	CAS No.	Regulation
A	MOCA	2,2'-dichloro-4,4'-methylenedianiline	101-14-4	REACH: authorisation
	MDA	4,4'- Diaminodiphenylmethane	101-77-9	REACH: authorisation
B	o-toluidine	o-toluidine	95-53-4	REACH: candidate for SVHC substance
	aniline	aniline	62-53-3	REACH: PACT-RMOA process completed
	diisocyanates (MDI/TDI)	methylene diphenyldiisocyanate; toluene diisocyanate	101-68-8 584-84-9 91-08-7	REACH, restriction proposal under consideration, binding OEL under discussion under EU Chemical Agents Directive (98/24/EC)
	paracetamol	N-acetyl-4-aminophenol	103-90-2	medicines regulations
C	p-PDA	p-phenylenediamine	106-50-3	REACH: PACT-RMOA process ongoing
	p-toluidine	p-toluidine	106-49-0	Registered under REACH, no other current regulatory actions
D		1,3-diphenylguanidine	102-06-7	Registered under REACH, subject for substance evaluation (CoRAP), decision available
		4,4-oxodianiline	101-80-4	REACH, candidate for SVHC
		N,N-diethylaniline	91-66-7	registered under REACH
		N-1-naphthylaniline	90-30-2	registered under REACH
		N-ethyl-N-[2-[1-(2-methylpropoxy)ethoxy]ethyl]-4-(phenylazo)aniline	34432-92-3	registered under REACH
		p-(2,3-epoxypropoxy)-N,N-bis(2,3-epoxypropyl)aniline m-(2,3-epoxypropoxy)-N,N-bis(2,3-epoxypropyl)aniline	5026-74-4 71604-74-5	registered under REACH
		1,1'-(p-tolylimino)dipropan-2-ol	38668-48-3	registered under REACH
	dapsone	80-08-0	registered under REACH	
E		other unspecified/unidentified aniline compounds	-	-

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6.3 Policy-related questions

Policy-related questions defined for the priority group “Anilines” are as follows:

1. What is the current occupational exposure to aniline and different aniline derivatives (including diamine forming diisocyanates) in the EU?
2. What is the exposure to paracetamol (aniline metabolite) among the general population?
3. What are the risks related to these exposures?
4. What is the possible impact of REACH on the exposure and risks?

6.4 Research Activities to be undertaken

As explained in this scoping document, “Anilines” is a large group of compounds. Therefore, it is suggested to focus on some priority compounds. These priorities are presented below. These have been selected largely on the basis of regulatory interests. Current information related to MOCA is considered sufficient and further research activities related to MOCA are not considered relevant.

Table 6-2: Listing of research activities to be carried out to answer the policy questions

Policy question No. (keyword)	Substance	Available knowledge related to policy question	Knowledge gaps / Activities needed to answer policy question
1, 2	MOCA	We have sufficient information on the toxicity and occupational exposure to MOCA. Validated biomonitoring methods are available in EU and information for the use of available biomarkers in occupational risk assessment.	No need for further research actions.
1, 2	4,4'-MDA	We have sufficient information on the toxicity and occupational exposure to 4,4'-MDA in the industrial use of this substance. Validated biomonitoring methods are available in EU and information for the use of available biomarkers in the risk assessment of occupational MDA exposure. There are only limited numbers of workers using 4,4'-MDA but exposure to 4,4'-MDA formed from methylene diphenyl diisocyanate may occur among the large group of workers and needs further studies (see below, item “diisocyanates”).	No need for further research actions related to the occupational exposure to 4,4'-MDA in its industrial use. Exposure to MDA in the use of methylene diphenyl diisocyanate (MDI), see below, item “diisocyanates”

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Policy question No. (keyword)	Substance	Available knowledge related to policy question	Knowledge gaps / Activities needed to answer policy question
1	aniline	Methods for the biomonitoring of aniline exist. Toxicity has been evaluated. Some biomonitoring data are available among general population and workers, however, data gaps exist. EU risk assessment concludes concern for workers, general population and consumers.	Risk assessment based on the available biomonitoring data for both workers and general population. Identification of the best biomarker for occupational and general population studies, paracetamol intake as confounder in the biomonitoring of aniline. Setting of reference and health based values. Bridging gaps related to exposure.
1	o-toluidine	Methods for biomonitoring exist. Toxicity has been evaluated. Limited biomonitoring data among general population and workers.	Bridging gaps related to the exposure of workers and general population. Risk assessment based on biomonitoring data. Setting of reference and health based values.
1	p-toluidine	Methods for biomonitoring exist. Toxicity has been evaluated. Only very limited biomonitoring data among general population and workers.	Bridging gaps related to the exposure of workers and general population. Risk assessment based on biomonitoring data. Setting of reference and health based values
1, 2	diisocyanates	Important causes of occupational asthma. Biomonitoring methods available but since asthma may occur at very low exposures, sensitivity of the methods should be high. Some occupational biomonitoring studies are available demonstrating exposure.	If/when restriction is going to become in force, there is a need to follow its effectiveness. Appropriateness/sensitivity of methods to detect low level exposures, still relevant for sensitisation. This may need further development. Characterisation of the all relevant exposure routes. Risk assessment and setting of limit values based on biomonitoring data.
1	paracetamol	There are general population biomonitoring data on paracetamol exposure available mainly from Denmark.	What is the general population exposure to paracetamol? Sources of the paracetamol exposure of general population. Paracetamol intake as a confounder in the biomonitoring of aniline. Identification of high exposures and risk assessment of exposure.
1	p-PPD	There are publications on the development of a method to measure exposure to p-PPD and testing of this method in hairdressers.	What is the exposure of general population and specific occupational groups, e.g. hairdressers to p-PPD, which is a common constituent of cosmetics and e.g. hair dyes.

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Policy question No. (keyword)	Substance	Available knowledge related to policy question	Knowledge gaps / Activities needed to answer policy question
1	anilines in general	Different aniline compounds may exist in various products or be formed as degradation of other products. Exposure may occur e.g. due to the pigment used in various products like hair dyes.	Screening of aniline exposure of general public and workers (including professionals like hairdressers), identification of compounds and sources of exposure. Identification of new biomarkers for anilines.

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7 Prioritised substance group: Aprotic solvents

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7.1 Background Information

In chemistry the solvents are qualitatively grouped into **non-polar, polar aprotic, and polar protic solvents**. A protic solvent is a solvent that has a hydrogen atom bound to oxygen (as in a hydroxyl group) or nitrogen (as in an amine group). In general terms, any solvent that contains a labile H⁺ is called a protic solvent. The molecules of such solvents readily donate protons (H⁺) to reagents. On the contrary, aprotic solvents cannot donate hydrogen as they do not have O-H or N-H bonds. The "a" means "without", and "protic" refers to protons or hydrogen atoms.

Examples of non-polar solvents are benzene, toluene, chloroform, dichloromethane, etc. Examples of polar protic solvents are water, most alcohols, formic acid, ammonia, etc. In their turn, some common polar aprotic solvents are acetone, acetonitrile, dimethylformamide, dimethylsulfoxide, etc.

There are numerous aprotic solvents, and they are widely used in different applications - as pH regulators and in water treatment products, anti-freeze products, coating products, lubricants and greases, adhesives and sealants, air care products (scented candles, air freshening sprays, electric and non-electric fragrance diffusers), non-metal-surface treatment products, inks and toners, leather treatment products, polishes and waxes, washing and cleaning products. They are also used as laboratory chemicals in scientific research, in agriculture, forestry and fishing as well as in the formulation of mixtures and/or re-packaging.

During the second prioritisation process within HBM4EU the ECHA and Germany proposed to include in the second list of priority substances four aprotic solvents that have a similar toxicological profile and a harmonised classification for reproductive toxicity:

- ▶ 1-methyl-2-pyrrolidone (NMP),
- ▶ 1-ethylpyrrolidin-2-one (NEP),
- ▶ N,N-dimethylacetamide (DMAC),
- ▶ N,N-dimethylformamide (DMF).

So, according to this proposal, the priority group of substances can be rephrased as "reprotoxic aprotic solvents".

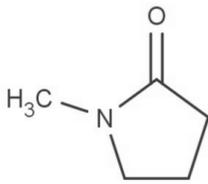
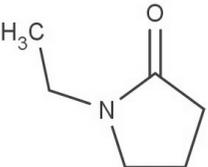
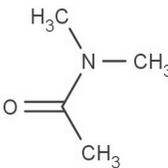
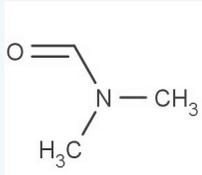
7.1.1 Hazardous properties

7.1.1.1 General characterisation

The toxicological profile of reprotoxic aprotic solvents is outlined in the Table 7-1.

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Table 7-1: Identification of HBM4EU prioritised reprotoxic aprotic solvents

Name	Abbreviation	CAS number	EC number	Formula	Structural formula	Harmonised classification acc. to CLP
1-methyl-2-pyrrolidone	NMP	872-50-4	212-828-1	C ₅ H ₉ NO		<i>Repr. 1B, H360D, STOT SE 3, H335 (C ≥ 10 %) Eye Irrit. 2, H319 Skin Irrit. 2, H315</i>
1-ethylpyrrolidin-2-one	NEP	2687-91-4	220-250-6	C ₆ H ₁₁ NO		<i>Repr. 1B, H360D</i>
N,N-dimethylacetamide	DMAC	127-19-5	204-826-4	C ₄ H ₉ NO		<i>Repr. 1B, H360D Acute Tox. 4, H332 Acute Tox. 4, H312</i>
N,N-dimethylformamide	DMF	68-12-2	200-679-5	HCON(CH ₃) ₂		<i>Repr. 1B, H360D Acute Tox. 4, H332 Acute Tox. 4, H312 Eye Irrit. 2, H319</i>

All substances in question have the harmonised classification according to PLC Regulation Repr. 1B, H360D (may damage the unborn child).

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7.1.1.2 Human toxicological information

A number of key animal studies providing information on reproductive toxicity of selected aprotic solvents are summarised below.

NMP

Teratogenicity studies were performed by Becci et al. (1982) in rats given N-methylpyrrolidone, Dosages of 75,237 and 750 mg of N-methylpyrrolidone/kg body weight/day were administered dermally to groups of 25 pregnant Sprague-Dawley rats on days 6 through 15 of gestation. Additionally, the study used a positive dermal control. Hexafluoroacetone, was chosen based on its dermal teratogenic activity. An oral positive control, aspirin, was included in order to add significance to the data generated in the experimental positive dermal control group. All animals were killed and subjected to uterine examination on day 20 of gestation. Maternal toxicity was indicated at 750 mg of N-methylpyrrolidone/kg by reduced body weight gain during gestation. Treatment with N-methylpyrrolidone resulted in dose-dependent brightly colored yellow urine and dry skin. Treatment at the high dosage level resulted in fewer live fetuses per dam, an increase in the percentage of resorption sites and skeletal abnormalities. These effects could be the result of maternal toxicity. There was no evidence of teratogenic effects nor effects on the dams at 75 and 237 mg/kg of body weight.

The developmental toxicity of N-methyl-2-pyrrolidone (NMP) was studied in Sprague–Dawley rats after oral administration (Saillenfait et al., 2002). Pregnant rats were given NMP at doses of 0, 125, 250, 500, and 750 mg/kg/day, by gavage, on gestational days (GD) 6 through 20. Significant decreases in maternal body weight gain and food consumption during treatment, and a reduction in absolute weight gain were observed at 500 and 750 mg/kg. The incidence of resorptions per litter was significantly higher than control at 500 mg/kg, and rose to 91% at 750 mg/kg. Examination of the foetuses revealed treatment-related malformations, including imperforate anus and absence of tail, anasarca, and malformations of the great vessels and of the cervical arches. The incidence of malformed foetuses per litter, and of litters with malformed foetuses was significantly increased at 500 and 750 mg/kg. There was a dose-related decrease in foetal body weights (male, female, and total) that reached statistical significance at 250 mg/kg. A significant increase in incomplete ossification of skull bones and of sternbrae was also present at 500 and 750 mg/kg. In summary, the no-observed-adverse-effect level (NOAEL) for maternal and developmental toxicity was 250 and 125 mg/kg/day, respectively. Thus, oral administration of NMP produced developmental toxicity below maternally toxic levels.

In addition, Saillenfait with coauthors studied the developmental toxicity of inhaled N-methyl-2-pyrrolidone (NMP) in Sprague–Dawley rats (Saillenfait et al., 2003). Pregnant rats were exposed whole body to NMP vapours at concentrations of 0, 30, 60 and 120 ppm, 6 h/day, on gestational days (GD) 6 through 20. Maternal body weight gain was significantly decreased at 60 and 120 ppm on GD 6–13 and maternal food consumption was reduced at 120 ppm on GD 13–21. No significant difference in the gestational weight change corrected for the weight of the gravid uterus was observed, whatever NMP concentration. There were no adverse effects on embryo/fetal viability or evidence of teratogenicity at any concentration tested. Fetal toxicity indicated by reduced fetal weight was observed at 120 ppm. Thus, the no-observed-adverse-effect level (NOAEL) for maternal and developmental toxicity was 30 and 60 ppm, respectively.

The relative embryotoxicity of the N-methyl-2-pyrrolidone (NMP) and its metabolites was evaluated using rat whole embryo culture (WEC) and the balb/c 3T3 cytotoxicity test (Flick et al., 2009). The resulting data were evaluated using two strategies; namely, one based on using all endpoints determined in the WEC and the other including endpoints from both the WEC and the cytotoxicity

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test. The substance with the highest embryotoxic potential is NMP, followed by 5-hydroxy-N-methyl-pyrrolidone (5-HNMP), 2-hydroxy-N-methylsuccinimide (2-HMSI) and N-methylsuccinimide (MSI). Specific dysmorphogeneses induced by NMP and 5-HNMP were aberrations in the head region of the embryos, abnormal development of the second visceral arches and open neural pores. Only NMP and 5-HNMP induced specific embryotoxic effects and were classified as weakly embryotoxic, whereas the other two metabolites, 2-HMSI and MSI, were determined to be non-embryotoxic.

NEP

The developmental toxicity of N-ethyl-2-pyrrolidone (NEP) was studied in Sprague-Dawley rats after oral administration (Saillenfait et al., 2007). Pregnant rats were given NEP at doses of 0, 50, 250, 500 and 750 mg/kg/day, by gavage (5 ml/kg), on gestational days (GD) 6–20. Maternal toxicity, as evidenced by reduction in body weight gain and food consumption, was observed in all NEP groups at the beginning of treatment (GD 6–9). The incidence of resorptions was significantly increased at 500 mg/kg/day, and reached 83% at 750 mg/kg/day. There was a dose-related decrease in fetal weight, which was significantly lower than control at 250 mg/kg/day and higher doses. The incidence of malformed fetuses per litter and the number of litters with malformed fetuses were significantly increased at 500 and 750 mg/kg/day. Malformations mainly consisted of edema, anal atresia with absent tail, cardiovascular defects and fused cervical arches. Ossification of skull bones and sternbrae was significantly reduced at 500 and 750 mg/kg/day. The incidence of supernumerary ribs was significantly elevated at 250 mg/kg/day and higher doses. The authors of the study made conclusion that NEP administered by gavage is embryotoxic and teratogenic at maternal toxic doses.

Additionally, NEP was evaluated in a 4-week repeated dose study in rats (Saillenfait et al., 2016). NEP diluted in distilled water was orally administered by gavage to male and female Sprague-Dawley rats at doses of 0, 5, 50, and 250 mg/kg/day for 28 consecutive days. Transient decreases in the body weight and in the body weight gain of the males was observed during the first days of treatment at the 50 and 250 mg/kg/day doses. There was a marked increase in urine volume at the beginning of treatment in males and female rats at doses of 50 and 250 mg/kg/day. No biologically significant differences were observed in hematological and clinical chemistry values in males and females at necropsy. Histological examination revealed an increase in hyaline droplets in the renal tubules of the kidneys and hepatocellular centrilobular hypertrophy in the liver of males at 250 mg/kg/day. Cytochrome P450 concentration in liver microsomes was slightly increased at 250 mg/kg/day in males. The results of this study demonstrate that NEP has mild to no effects at doses up to 250 mg/kg/day when administered orally to rats for 28 days with males being more susceptible than females.

With regard to human data, a number of publications are available, as well.

NMP

A 23-year-old laboratory technician was occupationally exposed to NMP during her first 20 weeks of pregnancy. The uptake via the lungs was probably of minor importance, as the NMP was handled at room temperature. Hand rinsing of glassware with NMP and cleaning up of an NMP spill in week 16 of pregnancy may have brought about a much larger uptake through the skin. During the 4 days following the spill, malaise, headache, and nausea were experienced. Examination of the pregnancy at week 14 showed no signs of delayed development; however, at week 25, signs of delayed fetal development were observed, and at week 31, a stillborn fetus was delivered. Stillbirth in this period of pregnancy is unusual. However, as the level of exposure is

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unknown, it is impossible to establish if exposure to NMP is the causative factor (Solomon et al., 1996; Bower, 1997).

A total of 15 24-h exposures in a repeated-insult patch test in human subjects (n = 50) caused minor to moderate transient irritations. No signs of contact sensitisation were observed. Direct contact of skin with NMP caused redness, swelling, thickening, and painful vesicles when NMP was used as a cleaner (Leira et al., 1992) or as a paint stripper (Åkesson & Jönsson, 2000).

Workers exposed to NMP in working areas with air concentrations up to 280 mg/m³ reported severe eye irritation and headache. With the methods of assessing the exposure level (sampling on charcoal and tracer gas method) and the response (observation and informal interview), it is impossible to develop a concentration–response relationship (Beaulieu & Schmerber, 1991). Six volunteers exposed to 10, 25, or 50 mg/m³ during 8 h in a chamber study registered their symptoms, before the start of exposure and then every 2 h for 16 h, in a questionnaire on a scale from 0 to 10 (0 = no symptoms and 10 = not tolerated). The volunteers displayed none of the following symptoms: eye or respiratory tract irritation; hacking cough, nose secretion, or blockage, sneezing, itching, or dryness in the mouth and throat, or other symptoms in upper airways; itching, secretion, smarting pain, visual disturbances, or other symptoms such as headache, dizziness, and nausea; and other symptoms. Two volunteers reported detecting an odour at 50 mg/m³. There were no significant differences in the spirometric data displayed by the forced expiratory volume in 1 s, vital capacity, and the highest forced expiratory capacity measured before or after any level of exposure. There were no acute changes in the nasal cavity assessed by continuous acoustic rhinometry. Even though the effects observed in this study were not very pronounced, it is mentioned that the possibility of undetected effects still remains (the number of volunteers was only six) (Åkesson & Paulsson, 1997).

In a dermal application experiment, 12 volunteers were exposed to 300 mg NMP through a dermal patch (filter paper, diameter 5 cm, protected with aluminium foil and attached by Dermalock) applied on the anterior face of the left forearm for 6 h. Five urine fractions were collected during 48 h following the onset of application. The mean dermal absorption of NMP was 67.9% (60.8 – 77.4%) (Ligocka et al., 2003).

NEP

No toxicity data in humans are available concerning NEP, however, it can be indicated that the toxicological profile of this substance is similar to NMP because both substances are structurally similar.

DMAC

No human data are available in relation to DMAC reproductive toxicity.

Liver toxicity was assessed in workers exposed to DMAC in an acrylic fiber manufacturing facility. Measurements were made over a 1-year study period. Evidence of liver toxicity was assessed by serum clinical chemistry tests (serum levels of total bilirubin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, & gamma-glutamyl transpeptidase) at least once during the study period for all 127 male workers in the two study departments and for 217 males in plant controls with no previous or current exposure to DMAC. Mean DMAC in air levels for the exposure groups appeared to differ (geometric mean DMAC in air levels of 1.9 and 1.3 ppm 12 hr TWA, respectively). No significant DMAC exposure related trends in hepatic serum clinical chemistry results were detected (Spies et al., 1995).

Two cases of toxic hepatitis from DMAC occurred among 25 employees on a new acrylic-fiber production line at a western U.S. manufacturing plant, probably due to inadequate personal

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protective equipment (PPE) for dermal exposures, resulting in skin penetration during maintenance and repair procedures. The authors concluded that hepatotoxicity due to dermal absorption of DMAC and other amide-type solvents deserves special consideration in industrial settings (Baum and Suruda, 1997).

Elastane fibre workers exposed to DMAC were monitored for hepatic injury. Four hundred and forty new workers employed from 1 January 2002 to 31 July 2004 were included as study subjects. DMAC exposure estimates were based on urinary N-methylacetamide concentrations. There were 28 cases of DMAC induced hepatic injury. The overall incidence of DMAC induced hepatic injury among new elastane fibre workers was 0.089/person-year. Incidence rates were 7–10 times higher in high exposure groups than in low exposure groups. Fewer DMAC induced hepatic injuries occurred among workers employed for a longer period. The inverse relation between the incidence of DMAC induced hepatic injury and duration of employment may reflect a type of healthy survivor effect or tolerance to DMAC induced hepatic injury (Lee et al., 2006).

DMF

Only one study is available on the reproductive effects of DMF in humans. This study reported an increased rate of spontaneous abortion among pregnant women occupationally exposed to DMF. However, these results cannot be attributed solely to DMF, as these women were exposed to a number of additional chemicals (U.S. EPA, 1986, 1999). 56 of 66 workers in a fabric coating factory participated in the study. All had standard liver function tests at least once. 46 workers completed a questionnaire; 27 had more extensive clinical evaluation for recognised liver abnormalities. An outbreak of toxic liver disease has been associated with exposure to DMF in the workplace. The diagnosis of toxic liver disease was established by the clinical histories, negative viral serologies, an enzyme pattern of alanine aminotransferase levels being greater than aspartate aminotransferase levels, epidemiologic data on coworkers, and liver biopsy specimens. The high prevalence of unsuspected liver enzyme abnormalities in these workers suggests that occupational liver disease may occur more frequently than is generally recognised (Redlich et al., 1988).

Chronic occupational exposure to DMF by inhalation has resulted in effects on the liver and digestive disturbances in workers. The Reference Concentration (RfC) for DMF was set 0.03 mg/m³ based on digestive disturbances and minimal hepatic changes suggestive of liver abnormalities in humans (U.S. EPA, 1999).

7.1.2 Exposure characteristics

7.1.2.1 Trends in production volume/environmental behaviour and concentrations

According to substance information given in the ECHA website, the tonnage bands of the 4 registered reprotoxic aprotic solvents in question are the following:

- ▶ NMP 10 000 – 100 000 t/year,
- ▶ NEP 1000 – 10 000 t/year,
- ▶ DMF 10 000 – 100 000 t/year,
- ▶ DMAC 10 000 – 100 000 t/year.

1 of 4 registrants of NEP under REACH indicated the substance as persistent, bio accumulative and toxic, not giving justification for it. In general, NMP, NEP, DMAC and DMF are considered to be readily biodegradable, non-persistent in the environment with low bioaccumulation potential

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(log Kow -0.11 for NMP and log Kow -0.11 for other three substances), according to US EPA (The EPI (Estimation Programs Interface) Suite™ KOWWIN™)¹.

Information on environmental concentrations is lacking.

7.1.2.2 Human related exposure sources and uses

Aprotic solvents including reprotoxic aprotic solvents in question are used as pH regulators and in water treatment products, anti-freeze products, coating products, lubricants and greases, adhesives and sealants, air care products (scented candles, air freshening sprays, electric and non-electric fragrance diffusers), non-metal-surface treatment products, inks and toners, leather treatment products, polishes and waxes, washing and cleaning products. They are also used as laboratory chemicals in scientific research, in agriculture, forestry and fishing as well as in the formulation of mixtures and/or re-packaging. Releases to the environment of the substances is likely to occur from indoor use (e.g. machine wash liquids/detergents, automotive care products, paints and coating or adhesives, fragrances and air fresheners), outdoor use, indoor use in close systems with minimal release (e.g. cooling liquids in refrigerators, oil-based electric heaters) and outdoor use in close systems with minimal release (e.g. hydraulic liquids in automotive suspension, lubricants in motor oil and break fluids). In addition, releases to the environment of those substances can occur from industrial use - manufacturing of the substance, formulation of mixtures, in processing aids at industrial sites and as an intermediate step in further manufacturing of another substance (use of intermediates).

Detailed information on possible uses and releases to environment can be found on ECHA web pages².

7.1.2.3 Human exposure routes

Both occupational exposure and exposure to the general public is relevant for reprotoxic aprotic solvents. Prevalence of high exposure is expected due to wide use and high production volume of substances under consideration. Exposure sources are ingredients in paints, graffiti remover, cleaning formulations, children's toys, textiles, carpets, inks, toner, pH-regulators, floccants, precipitants, neutralisation agents, laboratory chemicals, Especially regarding NMP and NEP, exposure of the general population since 1991 is confirmed (Ulrich et al., 2018).

Dermal exposure (possibly including cosmetic products containing aprotic solvents also) as well as inhalation exposure to indoor emissions from consumer products and articles mentioned is playing a role. It should be remarked that regarding the general public, reproductive toxicants category 1B shall not be placed on the market as substances, constituents of other substances or components of a mixture above 0.3 %.

Dermal exposure is considered to be especially significant. According to the *Opinion* of the Scientific Committee on Consumer Safety (SCCS) of EC on NMP adopted in 2011, NMP is readily absorbed by all routes of exposure, but, due to its low vapour pressure, absorption through the skin represents the most likely and potentially the most important route of exposure to NMP under most

¹ The EPI (Estimation Programs Interface) Suite™ is a Windows®-based suite of physical/chemical property and environmental fate estimation programs developed by EPA's and Syracuse Research Corp. (SRC). EPI Suite™ uses a single input to run the following estimation programs: KOWWIN™, AOPWIN™, HENRYWIN™, etc. (<https://www.epa.gov/tsca-screening-tools/epi-suite-estimation-program-interface>)

estimation programs: KOWWIN™, AOPWIN™, HENRYWIN™, etc. (<https://www.epa.gov/tsca-screening-tools/epi-suite-estimation-program-interface>)

² For NMP - <https://echa.europa.eu/brief-profile/-/briefprofile/100.011.662>;

For NEP - <https://echa.europa.eu/brief-profile/-/briefprofile/100.018.409>

For DMAC - <https://echa.europa.eu/brief-profile/-/briefprofile/100.004.389>

For DMF - <https://echa.europa.eu/brief-profile/-/briefprofile/100.000.617>

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known consumer use conditions. At the workplace, however, inhalation and dermal uptake can be assumed to be the important routes of exposure (*Scientific Committee on Occupational Exposure Limits* (SCOEL), SCOEL/REC/119, N-Methyl-2-Pyrrolidone, 2015).

For NEP, the German HBM Commission states that due to its use as substitute for NMP similar exposure routes can be assumed to be relevant for the human exposure, i.e. the inhalative and dermal exposure route (HBM Commission, 2015b).

SCCS in its conclusion in 2011 on NMP claims that based on a worst case assessment with a maximum use concentration of 5 % NMP in cosmetic products and a dermal absorption of 100 %, the Margin of Safety is considered to be too low. There is an absence of specific information on the actual possible maximum concentrations of NMP present in cosmetic products and specific measurement of dermal absorption of it through skin at relevant concentrations. With the information available at the time of assessment, the SCCS was of the opinion that the presence of NMP with a maximum use concentration of 5 % in cosmetic products is not safe for the consumer. A re-evaluation may be possible should relevant data that addresses the above be provided.

However, it is indicated that oral exposure through mists that deposit in the upper respiratory tract and are swallowed should be considered as well (US EPA, 2017). In addition, transplacental exposure shall be taken into account.

Professional and industrial workers, pregnant women and young children shall be considered as vulnerable sub-groups of population.

According to US EPA, for NMP adverse reproductive and other systemic effects could be a concern at higher exposures levels, but exposures that are protective of pregnant women and women who may become pregnant are expected to be also protective of other life stages and subpopulations (US EPA 2015).

7.1.2.4 Human Biomonitoring (HBM) data availability

The scientific publications concerning biomonitoring of reprotoxic aprotic solvents in question are listed in the References. As reprotoxic aprotic solvents are widely used as industrial chemicals, almost all available studies are performed in relation to occupational environment and/or very often in experimental settings with volunteers. Many of them were aimed at finding the appropriate exposure biomarkers and settings for biological monitoring in the occupational environment.

Only one study in relation to the general population and its sub-groups has been identified. **NMP** and **NEP** metabolite concentrations were determined in 540 24-h urine samples of the German Environmental Specimen Bank collected from 1991 to 2014. NMP metabolites 5-hydroxy-*N*-methyl-2-pyrrolidone (5-HNMP) and 2-hydroxy-*N*-methylsuccinimide (2-HMSI) as well as NEP metabolites 5-hydroxy-*N*-ethyl-2-pyrrolidone (5-HNEP) and 2-hydroxy-*N*-ethylsuccinimide (2-HESI) were determined by stable isotope dilution analysis using solid phase extraction followed by derivatisation (silylation) and GC–EI–MS/MS. The respective metabolites were identified: 5-HNMP in 98.0 % and 2-HMSI in 99.6% of the samples; 5-HNEP in 34.8 % and 2-HESI in 75.7% of the samples. Metabolite concentrations were rather steady over the timeframe investigated, even for NEP which has been introduced as an NMP substitute only in the last decade. Calculated median daily intakes in 2014 were 2.7 µg/kg bw/day for NMP and 1.1 µg/kg bw/day for NEP. For the combined risk assessment of NMP and NEP exposure, the hazard index based on the Human Biomonitoring assessment I values (HBM I values) was less than 0.1. Therefore, the individual and combined NMP and NEP exposures in Germany were within acceptable ranges in the investigated timeframe (Ulrich et al., 2018).

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NMP

Six female and six male volunteers (groups 1 and 2) were topically exposed for 6 hours to 300 mg of NMP. An additional group of six male volunteers (group 3) was exposed to 300 mg of NMP in a 50% water solution. Blood and urine were sampled before, during, and up to 9 days after the exposure. Plasma and urine were analysed using mass spectrometry. For groups 1 and 2, 16% and 18% of the applied dose were recovered in the urine as the sum of NMP and its metabolites. For group 3, 4% was recovered. The maximal concentration of 5-hydroxy-N-methyl-2-pyrrolidone (5-HNMP) was 10, 8.1, and 2.1 $\mu\text{mol/l}$ for groups 1, 2 and 3, respectively, in plasma and 420, 360 and 62 $\mu\text{mol/l}$ in urine adjusted for density.

For 2-hydroxy-N-methylsuccinimide (2-HMSI), the maximal concentration was 5.4, 4.5, and 1.3 $\mu\text{mol/l}$ for groups 1, 2 and 3, in plasma, respectively, and 110, 82 and 19 $\mu\text{mol/l}$ in urine adjusted for density. For 5-HNMP there was a difference in time to reach the maximal concentration depending on whether pure NMP or 50% NMP in water was used. No such difference was seen for 2-HMSI. The differences in kinetics between male and female volunteers were small. The authors concluded that preferably 2-HMSI should be used as the biomarker of exposure to NMP (Akesson et al., 2004).

Six male volunteers were exposed for 8 hours to NMP concentrations of 0, 10, 25, and 50 mg/m^3 . Blood and urine were sampled before, during, and up to 40 hours after exposure. Aliquots of urine and plasma were purified, derivatised, and analysed for 5-hydroxy-N-methyl-2-pyrrolidone (5-HNMP) on a gas chromatograph/mass spectrometer in the electron impact mode. The mean plasma concentration [P-(5-HNMP)] after 8-hour NMP exposure to 10, 25, and 50 mg/m^3 was 8.0, 19.6, and 44.4 $\mu\text{mol/l}$, respectively. The mean urinary concentration [U-(5-HNMP)] for the 2 last hours of exposure was 17.7, 57.3, and 117.3 mmol/mol creatinine, respectively.

The maximal P-(5-HNMP) and U-(5-HNMP) concentrations occurred 1 hour and 0-2 hours, respectively, after the exposure. The half-lives of P-(5-HNMP) and U-(5-HNMP) were 6.3 and 7.3 hours, respectively. The 5-HNMP urinary concentrations were 58% of the calculated retained dose. There was a close correlation (r) between P-(5-HNMP) ($r=0.98$) and U-(5-HNMP) ($r=0.97$) with NMP exposure. The authors concluded that 5-HNMP as biomarker in plasma is recommended (Akesson et al., 2000).

An experimental study with 16 volunteers exposed to 80 mg/m^3 NMP for 8 h under either whole-body, i.e. inhalational plus dermal, or dermal-only conditions was carried out. Additionally, the influence of moderate physical workload on the uptake of NMP was studied. The urinary concentrations of NMP and its metabolites 5-hydroxy-N-methyl-2-pyrrolidone (5-HNMP) and 2-hydroxy-N-methylsuccinimide (2-HMSI) were followed for 48 h and analysed by gas chromatography-mass spectrometry (GC-MS). Percutaneous uptake delayed the elimination peak times and the apparent biological half-lives of NMP and 5-HNMP. Under resting conditions, dermal-only exposure resulted in the elimination of 71 +/- 8 mg NMP equivalents as compared to 169 +/- 15 mg for whole-body exposure. Moderate workload yielded 79 +/- 8 mg NMP (dermal-only) and 238 +/- 18 mg (whole-body). Thus, dermal absorption from the vapour phase may contribute significantly to the total uptake of NMP, e.g. from workplace atmospheres (Bader et al., 2008).

The study by Haufroid et al. (2014) was performed in order to examine the value of urinary 5-hydroxy-N-methyl-2-pyrrolidone (5-HNMP) and 2-hydroxy-N-methylsuccinimide (2-HMSI) in a population of workers exposed to N-methyl-2-pyrrolidone (NMP) and to look for health effects of exposure to this organic solvent. Airborne NMP was determined according to the NIOSH method. Urinary 5-HNMP and 2-HMSI (after and before next shift) were determined by liquid chromatography with tandem mass spectrometry. Outcomes were effects on lung, kidney, skin and

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mucous membranes, nervous system, haematopoiesis and liver determined by clinical examination and laboratory measurements. Univariate statistical methods and multiple regressions were used to analyse results. Skin resorption, smoking and other potential confounders were taken into account. 327 workers were eligible out of which 207 workers (63%) participated. 91 of these worked with NMP. Occupational exposure to NMP did often not occur daily and ranged from non-detectable to 25.8 mg/m³ (median = 0.18). Urinary 2-HMSI (mg/l; before next shift) was the best biomarker of exposure to NMP, explaining about 70% of the variance, but most likelihood ratios did not allow for ruling exposure in or out, at these low levels of exposure. Creatinine adjustment did not improve the results clearly. No clear and consistent health effects could be associated with NMP exposure. No indication for a bias due to non-participation was found. The authors stated that biological monitoring, primarily urinary 2-HMSI (mg/l; before next shift), is of value to estimate exposure to NMP even when exposure is irregular and low. Likelihood ratios of urinary 5-HMNP or 2-HMSI are, however, not quite satisfactory at these low levels. No irritant or other health effects were found.

Meier et al (2013) reported on a study investigating current exposures to NMP in the spraying department of an automobile plant using biological monitoring. 5-HNMP and 2-HMSI were analysed in 69 urine samples of 14 workers exposed to NMP and of 9 non-exposed controls. Measurements of airborne exposure levels were not included. Three different working tasks ('loading' and 'cleaning' of the sprayer system and 'wiping/packing' of the sprayed materials) and three sampling times (pre-shift, post-shift, and pre-shift of the following day) were studied in exposed workers.

Median levels of 5-HNMP and 2-HMSI in post-shift urine of exposed workers were 0.91 and 0.52 mg/g creatinine, respectively, whereas median levels in controls were below the limit of detection. Decreased levels of 5-HNMP were observed in pre-shift urine samples on the following day (0.39 mg/g creatinine) in exposed workers, while the concentration of 2-HMSI did not change (0.49 mg/g creatinine). Highest exposures occurred during sprayer cleaning with a maximum level of 8.31 mg/g creatinine of 5-HNMP in post-shift urine. In contrast to 'wipers/packers', no decrease in 5-HNMP could be observed in pre-shift urine samples on day 2 of the 'loaders' and 'cleaners'. Overall, exposure in terms of 5-HNMP post-shift and 2-HMSI pre-shift of the following day were well below existing biological limit values of the European Union (70 and 20 mg/g creatinine, respectively). The authors suggested that the analysis of 5-HNMP in pre-shift samples also provided essential information, particularly in situations involving direct handling of liquid NMP-containing formulations.

NEP

Koch et al. (2014) orally dosed 20.9 mg NEP to three male volunteers. These volunteers collected all their urine samples over a period of 4 days post dose. In these samples NEP metabolites 5-hydroxy-N-ethyl-2-pyrrolidone (5-HNEP) and 2-hydroxy-N-ethylsuccinimide (2-HESI) were identified and quantified, and their urinary elimination kinetics and their metabolic conversion factors were determined. After 4 days the researchers recovered 50.7 % of the dose of these two metabolites in urine, 29.1 % of 5-HNEP and 21.6 % of 2-HESI. The largest share of 5-HNEP was excreted within 24 h post dose, while the major share of 2-HESI was excreted on day 2 post dose. An elimination half-time for 5-HNEP of approx. 7 h and for 2-HESI of approx. 22-27 h was estimated. While the elimination of 5-HNEP was basically finished 72 h post dose, significant amounts of 2-HESI were still eliminated after 96 h. Both biomarkers can be used in Human Biomonitoring studies to extrapolate from urinary measurements to the NEP dose taken up and thus to evaluate the risk caused by exposure to this chemical.

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DMAC

Worker exposure to DMAC in an acrylic fiber manufacturing facility was measured, over a 1-year study period, by full-shift (12 hours) personal air monitoring for DMAC and by biological monitoring for levels of DMAC, N-methylacetamide (MMAC), and acetamide in spot urine samples. Ninety-three of 127 male workers in seven job classifications in the solution preparation and spinning departments of the plant were monitored on the second consecutive workday after at least 3 days off for the first 10 months of the study and on both the first and second days during the study's final 2 months. Postshift urinary MMAC levels were significantly correlated ($P < .0001$, $r_2 = .54$) with DMAC in air levels. An air level of 6.7 ppm 12-hour time-weighted average (TWA) corresponded to a urine MMAC level of 62 mg/g creatinine in a postshift spot urine sample obtained after the second consecutive workday. To minimise exposure misclassification due to variability in the regression relationship, a level of 35 mg MMAC/g creatinine in a postshift spot urine sample was recommended as a biomonitoring index. Postshift urine MMAC levels did not appear to plateau at higher air levels, nor did it appear that the DMAC demethylation metabolic mechanisms became saturated at threshold limit value (TLV)-level air-exposure levels. Urine MMAC levels in postshift samples obtained the second workday appeared to be greater than levels in postshift first-day samples, but the number of days until this postshift level would plateau could not be determined from this study (Spies et al., 1995).

Perbellini et al (2003) studied the concentration of N,N-dimethylacetamide (DMAC) and its metabolite, N-methylacetamide (NMA), in urine of workers occupationally exposed to DMAC in a factory producing synthetic acrylic fibres. During the first phase, 223 workers exposed to low environmental concentrations of DMAC provided urine samples at the end of a work shift. High concentrations of the unmodified solvent and its metabolite were found in a group of workers whose job was to start up machinery. The second and third phases focused on conditions favouring high uptake of DMAC. The highest concentrations of unmodified solvent and NMA were found in the urine of workers recently engaged in starting up machinery. NMA in urine was 1.5-173.6 mg/g creatinine (median 20.5). In spite of the low environmental concentration, about 20% of the urine concentration of NMA was higher than 30 mg/g creatinine. Dermal absorption of DMAC was high. A shower and a change of clothing at the end of the work shift, and washing away any solvent left on the skin, ensured that dermal absorption of DMAC did not continue. This significantly reduced the NMA urinary concentration at values lower than 30 mg/g creatinine. In some urine samples, S-acetamidomethyl-mercapturic acid (AMMA) was identified by NMR analysis; this is probably a metabolite of N,N-dimethylacetamide--it has never before been identified in humans or animals. The authors remarked that even at low environmental concentrations of DMAC, dermal absorption can be considerable. Unmodified DMAC and NMA concentrations in urine are good biomarkers for monitoring occupational exposure to the solvent.

Princivalle et al (2010) studied toxicokinetics of two major urinary metabolites of DMAC namely, S-(acetamidomethyl)mercapturic acid (AMMA) and N-methylacetamide (NMA). Urine samples were collected from workers exposed to DMAC in a factory manufacturing acrylic fibers. AMMA and NMA were determined by HPLC/MS and GC/MS, respectively. The working scheme in the factory consisted of periods of three consecutive working shifts alternated regularly with two days off work. In the first stage of the study, NMA and AMMA were determined in urine samples collected before, in the middle, and at the end of one working shift. In the second stage, urine was collected five times during three consecutive days after a two-day rest: before and at the end of the first and second working shifts and before the third shift. It was found that the end-of-shift NMA levels were several folds higher than the pre-shift levels of the same day and dropped significantly until the next shift. On the other hand, there were no significant differences in AMMA levels before and at the end of the same shift but a continuous rise during the three-day working period was observed.

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Median values of NMA concentrations at the end of working shifts were between 10.1 and 17.3 mg/g creatinine, median AMMA concentrations in the second or third day of the working period varied between 12.4 and 38.1 mg/g creatinine. The approximate half-lives of NMA and AMMA (means) in the exposed workers were about 9 and 29 h, respectively. Thus, while NMA in the end-of-shift urine samples remains a preferential biomarker of DMAC exposure during that shift, AMMA determined at the end of a work-week reflects cumulative exposure over the last few days. The authors made conclusion that further studies are needed to determine AMMA concentrations corresponding to the threshold limit value of DMAC.

DMF

DMF exposure was monitored in a synthetic leather factory; at the same time, urinary DMF and its metabolites were measured in urine samples collected before and at the end of workshifts. The study was run during two different periods. During the first phase ten workers were observed for 3 days (Monday, Tuesday and Wednesday) in the same week. In the second phase 16 workers were involved in the study on a Friday and on the following Monday.

Urinary DMF, as well as hydroxymethyl-N-methylformamide and hydroxymethylformamide [measured as N-methylformamide (NMF) and formamide, respectively], were measured as a "physiological" product in subjects not exposed to dimethylformamide. Environmental exposure to DMF ranged between 10 and 25 mg/m³.

The unmodified solvent found in urine collected at the end of the exposure was significantly related to the environmental concentrations of DMF; its urinary concentrations were found to range between 0.1 and 1 mg/l. Higher concentrations of NMF (mean 23.3 mg/l) and formamide (24.7 mg/l) were measured in urine samples collected at the end of workshifts. The same concentrations were related to individual exposures to DMF. N-Acetyl-S-(N-methylcarbamoyl)cysteine (AMCC) in the urine of workers exposed to DMF showed a mean concentration of 40.4 mg/l on Friday (before and after the workshift) and a mean concentration of 10.3 mg/l on Monday. Its slow kinetic profile favours its body accumulation during the working week (Lareo and Perbellini, 1995).

To estimate the contribution of skin absorption to total body burden of DMF across a working week in two groups with similar levels of respiratory exposure but dissimilar skin contact 25 workers in a synthetic leather (SL) factory, 20 in a copper laminate circuit board (CLCB) factory, and 20 age and sex matched non-DMF exposed subjects, were recruited. Environmental monitoring of DMF exposure via respiratory and dermal routes, as well as biological monitoring of pre-shift urinary N-methylformamide (U-NMF), were performed for five consecutive working days. Environmental and biological monitoring showed no detectable exposure in controls. The average airborne DMF concentration (geometric mean (GM) 3.98 ppm, geometric standard deviation (GSD) 1.91 ppm), was insignificantly lower for SL workers than for CLCB workers (GM 4.49, GSD 1.84 ppm). Dermal DMF exposure and U-NMF values, however, were significantly higher for SL workers. A significant pattern of linear accumulation was found across a five day work cycle for SL workers but not for CLCB workers. Dermal exposure to DMF over five consecutive days of occupational exposure can result in the accumulation of a significant DMF body burden (Chang et al., 2005).

In order to measure exposure to DMF in occupational settings in 35 healthy workers employed in the polyacrylic fibre industry, N-methylformamide (NMF) and N-acetyl-S-(N-methylcarbamoyl)cysteine (AMCC) in urine, and N-methylcarbamoylated haemoglobin (NMHb) in blood were measured. Workplace documentation and questionnaire information were used to categorise workers in groups exposed to low, medium, and high concentrations of DMF. All three biomarkers can be used to identify occupational exposure to DMF. However, only the analysis of NMHb could accurately distinguish between workers exposed to different concentrations of DMF.

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The median concentrations were determined to be 55.1, 122.8, and 152.6 nmol/g globin in workers exposed to low, medium, and high concentrations of DMF, respectively. It was possible by the use of NMHb to identify all working tasks with increased exposure to DMF. While fibre crimpers were found to be least exposed to DMF, persons washing, dyeing, or towing the fibres were found to be highly exposed to DMF. In addition, NMHb measurements were capable of uncovering working tasks, which previously were not associated with increased exposure to DMF; for example, the person preparing the fibre forming solution. Measurement of NMHb in blood is recommended rather than measurement of NMF and AMCC in urine to accurately assess exposure to DMF in health risk assessment. However, NMF and AMCC are useful biomarkers for occupational hygiene intervention (Kafferlein et al., 2005).

Seitz et al. (2018) assessed the relation between occupational exposure to DMF after an 8 h work shift in the acrylic fibre industry and its three biological markers N-methylformamide (NMF_{total}), N-acetyl-S-(N-methylcarbamoyl)cysteine (AMCC), and N-methylcarbamoyl adduct at haemoglobin (MCVal). External DMF exposure of 220 workers was determined during the whole shift. A standardised questionnaire was used to obtain information about the worker's general health status, medical treatment, smoking habits, protective measures, and possible symptoms caused by DMF exposure. NMF and AMCC were analysed in post-shift urine samples and MCVal in blood. For longitudinal assessment the average AMCC concentration was determined over a period of 4 weeks (weekly sampling) in a sub-collective of 89 workers. The median of DMF concentration in air was 3.19 mg/m³ (range < 0.15-46.9 mg/m³).

The biological markers showed a median of 4.80 mg/L (range 0.20-50.6 mg/L) for NMF_{total}, 4.75 mg/g creatinine (range 0.06-49.6 mg/g creatinine) for AMCC, and 57.5 nmol/g globin (range 0.5-414 nmol/g) for MCVal. A significant linear relationship was observed between DMF in air and NMF as well as between DMF in air and AMCC in post-shift urine samples. The mean AMCC values measured weekly over a period of 4 weeks correlated significantly with MCVal adducts too. Excluding workers who had been using breathing masks on the day of the study led to even tighter correlations. The results of the present study demonstrate the applicability of the DMF biomonitoring parameters NMF_{total} in post-shift urine for the present-day exposure assessment, AMCC in the post-shift urine after several shifts for assessment of the cumulative exposure of the previous working days, and MCVal for assessment of long-term exposure during previous weeks and months.

7.1.2.5 Health based guidance values available for HBM data

For the protection of the general population, the German Environment Agency (Umweltbundesamt; UBA) recommends two types of health-based guidance values for **NMP** expressed as the sum of the concentration of two main metabolites of NMP in urine - 5-hydroxy-NMP and 2-hydroxy-N-methylsuccinimide (2-HMSI) and for NEP expressed as the sum of the concentration of two main metabolites of NEP in urine – 5-hydroxy-NEP (5-HNEP) and 2-hydroxy-n-ethylsuccinimide (2-HESI):

- ▶ NMP: control values (HBM-I) of 10 mg/L for children and 15 mg/L for adults
- ▶ NMP: action values (HBM-II) of 30 mg/L for children and 50 mg/L for adults

Exposure to NEP can be quantified by the determination of the excretion of its urinary metabolites 5-Hydroxy-N-ethyl-2-pyrrolidone (5-HNEP) and 2-Hydroxy-N-ethylsuccinimide (2-HESI). The resulting HBM-I and HBM-II values for the sum of the metabolites 5-HNEP and 2-HESI in the urine are the following:

- ▶ NEP: control values (HBM-I) of 10 mg/L for children and 15 mg/L for adults
- ▶ NEP: action values (HBM-II) of 25 mg/L for children and 40 mg/L for adults

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For workers exposure to **NMP** a BLV of **20 mg/g creatinine** expressed as urine concentration 18 hours after the end of the previous exposure (pre-shift sample) for the 2-HMSI is established.

As NMP and NEP act very similar, UBA suggests using a mixture approach considering the sum of the 4 metabolites when a combined exposure to both compounds is expected. (Kommission Human Biomonitoring, 2015a)

As regards **DMAC**, BLV for workers of **20 mg/g creatinine** expressed as N-methylacetamide (NMAC) concentration in urine at the end of shift at the end of workweek is proposed (Qian YL et al., 2012)³.

In their turn, The American Conference of Governmental Industrial Hygienists (ACGIH®)⁴ recommends a Biological exposure index (BEI) of **30 mg/g creatinine** for NMAC concentration in urine at the end of shift at the end of the work week samples as reference value for workers, and the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) recommends the same limit value of **30 mg/g creatinine** in relation to NMAC urine concentration at the end of shift at the end of the work week (Perbellini L et al., 2003)⁵.

According to the SCOEL, for occupational exposure to **DMF** the BLV expressed as N-methylformamide (NMF) concentration in urine **15 mg/L** at the end of shift at the end of work week is established. In its turn, the ACGIH's Biological Exposure Index for the same metabolite is **30 mg/L** (end of shift, end of the work week).

7.1.3 Policy relevance

7.1.3.1 Existing regulation (sectoral and inter-sectoral policies)

All 4 aprotic solvents in question are classified as Repr.1B, H360D (May damage the unborn child) according to CLP Regulation. Regarding general public and according to the entry 30 of Annex XVII of REACH, reproductive toxicants category 1B shall not be placed on the market as substances, constituents of other substances or components of a mixture above 0.3 %.

In addition, with respect to consumer protection DMAC and DMF are listed in Annex II (list of substances prohibited in cosmetic products – entries 747 and 355, respectively) of the Cosmetic Products Regulation No1223/2009.

NMP, DMAC and DMF are used in the production of medicinal products and are therefore subject to the provisions of directives 2001/83/EC on medicinal products for human use and 2001/82/EC on veterinary medicinal products, as well as those of Commission Delegated Regulation (EU) No 1252/2014 on principles and guidelines of good manufacturing practice for active substances for medicinal products for human use.

NMP, DMAC and DMF are listed on the Candidate List under REACH as SVHC and included in the candidate list for authorisation.

Some uses of the NMP are restricted under Annex XVII of REACH, namely, the following conditions for restriction are set:

- ▶ Shall not be placed on the market as a substance on its own or in mixtures in a concentration equal to or greater than 0,3 % after 9 May 2020 unless manufacturers, importers and downstream users have included in the relevant chemical safety reports and safety data

³ <https://www.ncbi.nlm.nih.gov/pubmed/23257108>

⁴ <https://www.acgih.org/>

⁵ https://www.researchgate.net/publication/9085557_Biological_monitoring_of_occupational_exposure_to_NN-dimethylacetamide_with_identification_of_a_new_metabolite

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sheets, Derived No-Effect Levels (DNELs) relating to exposure of workers of 14,4 mg/m³ for exposure by inhalation and 4,8 mg/kg/day for dermal exposure (paragraph 1).

- ▶ Shall not be manufactured, or used, as a substance on its own or in mixtures in a concentration equal to or greater than 0,3 % after 9 May 2020 unless manufacturers and downstream users take the appropriate risk management measures and provide the appropriate operational conditions to ensure that exposure of workers is below the DNELs specified in paragraph 1 (paragraph 2).
- ▶ By way of derogation from paragraphs 1 and 2, the obligations laid down therein shall apply from 9 May 2024 in relation to placing on the market for use, or use, as a solvent or reactant in the process of coating wires (paragraph 3).

Within assessment of restriction proposal for NMP ECHA Risk Assessment Committee (RAC) has set a DNEL value for NMP **10 mg/m³** (chronic inhalation exposure for workers covering pregnant women)⁶. A dermal DNEL of **4.8 mg/kg/day** is also proposed by RAC for the workers.

The European Commission adopted the restriction for NMP on 18 April 2018⁷.

Furthermore, ECHA RAC and Committee for Socio-economic Analysis (SEAC) adopted their Opinion on REACH Annex XV dossier proposing restrictions on DMF in September 2019 and December 2019, respectively. The following conditions for restriction are set:

- ▶ Manufacturers, importers and downstream users of the substance on its own (regardless of whether DMF is a (main) constituent, an impurity or a stabiliser) or in mixtures in a concentration equal or greater than 0.3 % shall use in their chemical safety assessment and safety data sheets by [xx.yy.zzzz] a worker based harmonised Derived No Effect Level (DNEL) value for long-term inhalation exposure of 6 mg/m³ and a worker based harmonised DNEL for long-term dermal exposure of 1.1 mg/kg bw/day.

Similarly to the restriction on NMP, to enable biomonitoring, RAC recommends to derive a DNEL(biomarker) since DMF can be readily absorbed via exposed skin. RAC noted that biomonitoring is not needed for REACH enforcement.

In addition, DMF is considered as Category 2A substance with respect to carcinogenicity according to IARC⁸ and many organic solvents are mentioned as neurotoxicants (Grandjean, 2006; US EPA 2015).

Furthermore, DMF is included in the priority list of chemicals developed within the EU-Strategy for Endocrine Disruptors and placed in category 3 - no evidence of endocrine disrupting activity or no data available and listed in Annex 13 (List of 146 substances with endocrine disruption categorisations prepared in the Expert meeting)⁹.

According to Directive 2010/75/EU of 24 November 2010 on industrial emissions (integrated pollution prevention and control), substances with CMR (carcinogenic, mutagenic, or toxic for reproduction) properties shall be replaced as far as possible by less harmful substances or mixtures within the shortest possible time.

⁶ <https://echa.europa.eu/documents/10162/aa77c7c4-4026-4ab1-b032-8a73b61ca8bd>

⁷ <https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32018R0588&from=EN>

⁸ Volume 47, 71, 115 (In prep.)

⁹ http://ec.europa.eu/environment/chemicals/endocrine/strategy/substances_en.htm

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According to Directive 98/24/EC of 7 April 1998 on the protection of the health and safety of workers from the risks related to chemical agents at work, employers are required to eliminate risks or reduce them to a minimum, with a preference for substitution.

For the workers the Occupational exposure limits (OELs) are set for the following reprotoxic aprotic solvents in question:

- ▶ NMP: **40 mg/m³** or **10 ppm** (8-hours TWA) and **80 mg/m³** or **20 ppm** (short term);
- ▶ DMAC: **36 mg/m³** or **10 ppm** (8-hours TWA) and **72 mg/m³** or **20 ppm** (short term);
- ▶ DMF: **15 mg/m³** or **5 ppm** (8-hours TWA) and **30 mg/m³** or **10 ppm** (short term).

It should be remarked that different national limits may be lower.

In addition, on 23 September 2015, SCOEL confirmed their recommendation of 2007 for an OEL time-weighted average (TLV-TWA¹⁰) of **10 ppm (40 mg/m³)**, a short-term exposure limit (TLV-STEL¹¹) of **20 ppm (80 mg/m³)** and a BLV of **70 mg/g creatinine** in urine for 5-hydroxy-N-methyl-2-pyrrolidone (5-HNMP), monitored 2-4 h after exposure/shift with a supplemented 'skin' notation and adopted the revised Recommendation SCOEL/REC/119¹².

In addition with respect to the general population, the US EPA has proposed a Reference concentration (RfC)¹³ for DMF **30 µg/m³** (last revised 10/01/1990¹⁴).

7.1.3.2 Upcoming regulation

ECHA RAC will start to evaluate the DMAC restriction proposal in 2020 – 2021. NEP is likely to be considered for restriction in the future.

7.1.4 Technical aspects

7.1.4.1 Biomarkers available for parent compounds or metabolites in human matrices

A metabolic pathway suggested for humans is the following: **NMP** is first hydroxylated to 5-hydroxy-N-methyl-2-pyrrolidone (5-HNMP), and then oxidised to N-methylsuccinimide (MSI), which in turn is hydroxylated to 2-hydroxy-N-methylsuccinimide (2-HMSI) (Figure 7.1).

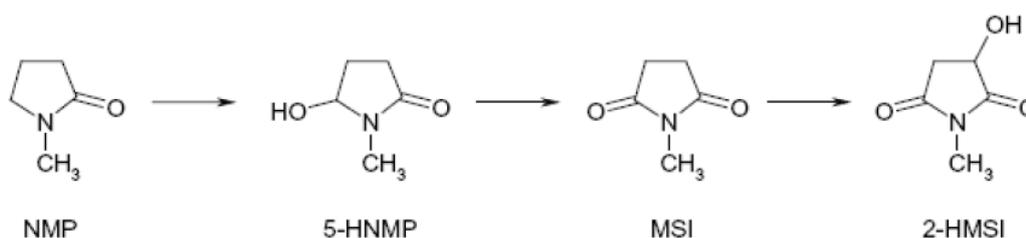


Figure 7.1: Proposed metabolism of NMP (Akesson et al., 1997; Carnerup et al., 2005)

It is stressed that main metabolites of **NMP** are 5-hydroxy-NMP and 2-hydroxy-N-methylsuccinimide (2-HMSI) in urine (Apel et al., 2017). 2-HMSI is suggested as a biomarker of exposure to NMP, and the levels in plasma and urine may be used to indicate an exposure over three days as the half-life of 2-HMSI is longer than for the other metabolites (Jönsson et al., 2003).

¹⁰ Threshold limit value - Time-weighted average

¹¹ Threshold limit value - Short term exposure limit

¹² http://files.chemicalwatch.com/2016-03-30_SCOEL-OPIN-2016-119.pdf

¹³ The RfC is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily inhalation exposure of the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime (US-EPA)

¹⁴ https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0511_summary.pdf

As regards **NEP**, similarly to NMP, the main metabolites are 5-hydroxy-NEP and 2-hydroxy-N-ethylsuccinimide (Koch et al., 2014).

For the **DMAC** the main metabolites are N-methylacetamide (NMAC), N-hydroxymethylacetamide, acetamide (DMAC-OH) and N-acetyl-S-(acetamidomethyl)-L-cysteine (AMMA) (Figure 7.2). According to French National institute of research and security (INRS) and its Biotox database¹⁵, urine acetamide as a marker of DMAC exposure has been proposed but it is less well correlated with atmospheric DMAC levels in occupational environment than urinary NMAC. The determination of AMMA in urine at the end of the work week is considered to be interesting for biological monitoring of occupational exposure. Concentrations of the order of 10 to 40 mg/g creatinine are found in employees whereas NMAC levels are around 10 to 17 mg/g creatinine. BLV for workers is set for NMAC (see information provided above).

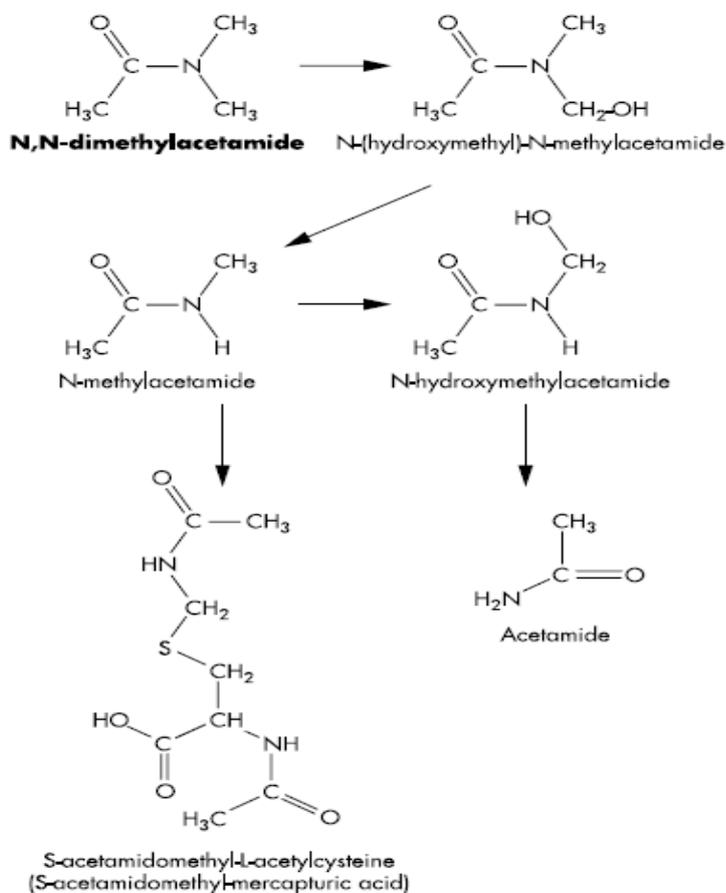


Figure 7.2: Proposed metabolism of DMAC (Perbellini et al., 2003)

¹⁵ Biotox database is a biological monitoring guide for occupational physicians that is used in the health surveillance of exposed workers. It covers over 100 chemical substances and specifies the marker, the metabolic background with the influencing factors, and the biological medium to be sampled (<http://www.inrs.fr/publications/bdd/biotox.html>).

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DMF main metabolites are N-methylformamide (NMF), N-hydroxymethylformamide (HMMF), acetamide and N-acetyl-S-(acetamidomethyl)-L-cysteine (AMCC) (Figure 7.3).

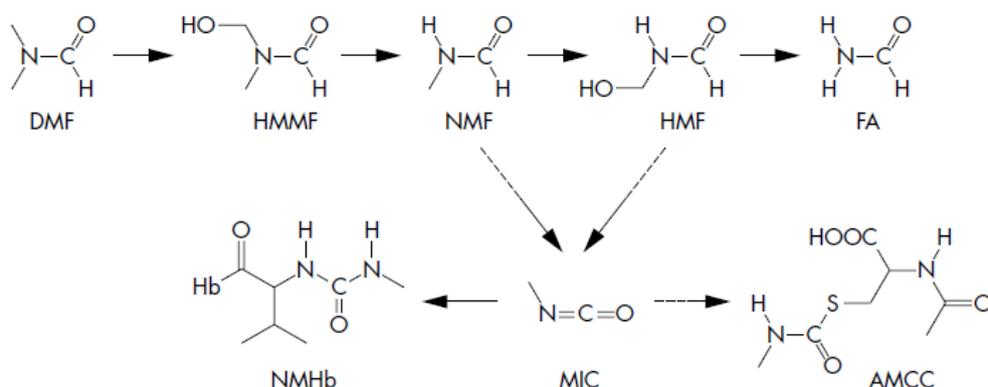


Figure 7.3: Proposed metabolism of DMF (Kafferlein et al., 2005)

Notes: N-hydroxymethyl-N-methylformamide (HMMF); N-methylformamide (NMF); N-hydroxymethylformamide (HMF); formamide (FA); methyl isocyanate (MIC); N-methylcarbamoylated haemoglobin (NMHb); N-acetyl-S-(N-methylcarbamoyl)cysteine (AMCC)

7.1.4.2 Main characteristics of analytical methods

Gas chromatography-mass spectrometry was used for quantitative analysis of urine samples in relation to NMP (Bader M. et al., 2006) and cooled-injection gas chromatography and isotope dilution mass spectrometry is used to quantify all four metabolites of NMP and NEP (Schindler et al., 2012).

It can be assumed that gas chromatography-mass spectrometry is applicable for determination of other metabolites associated to DMAC and DMF as well. However, it is indicated that the precision of traditional gas chromatography is low due to the thermal decomposition of metabolites in the high-temperature gas chromatography injection port. To overcome this problem, a new method for the simultaneous separation and quantification of urinary DMAC metabolites using liquid chromatography-tandem mass spectrometry is developed (Yamamoto S. et al., 2018)¹⁶.

As the biomarker of DMAC - DMAC-OH is decomposed during gas chromatography analysis, the total concentration of NMAC is the sum of DMAC-OH and NMAC. The same consideration is relevant to the biomarker of DMF – HMMF which will be decomposed during gas chromatography procedure as well. Therefore the total concentration of NMF is the sum of HMMF and NMF.

7.1.5 Societal concern

NMP, NEP, DMAC and DMF are listed in the SIN List. The SIN (Substitute It Now!) List is a comprehensive database of chemicals likely to be restricted or banned in the EU. It is publicly available, regularly updated and provided completely free of charge by non-profit organisation ChemSec (<https://chemsec.org>).

NMP, DMAC and DMF are included in the Trade Union Priority List for REACH authorisation.

¹⁶ <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5886881/>

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7.2 Categorisation of Substances

Table 7-2: Substances included in the substance group, listed according to availability of toxicology and human biomarker data, in category A, B, C, D, E substances (see general introduction)

Category	Abbreviation/ Acronym	Systematic name	CAS No.	Regulation
B	NMP	1-methyl-2-pyrrolidone	872-50-4	REACH: SVHC, included in the candidate list for authorisation, restricted under Annex XVII CLP: harmonised classification Repr. 1B, H360D
	DMF	N,N-dimethylformamide	68-12-2	REACH: SVHC, included in the candidate list for authorisation CLP: harmonised classification Repr. 1B, H360D Cosmetic Products Regulation: listed in Annex II - substances prohibited in cosmetic products
C	DMAC	N,N-dimethylacetamide	127-19-5	REACH: SVHC, included in the candidate list for authorisation CLP: harmonised classification Repr. 1B, H360D Cosmetic Products Regulation: listed in Annex II - substances prohibited in cosmetic products
D	NEP	1-ethylpyrrolidin-2-one	2687-91-4	CLP: harmonised classification Repr. 1B, H360D

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7.3 Policy-related questions

1. What is the current external exposure of the workers in EU to reprotoxic aprotic solvents and do they exceed Guidance values (reference values), where they are available? What data gaps exist?
2. What is the current internal exposure of the workers in EU to reprotoxic aprotic solvents, especially with respect to female workers at reproductive age, and do they exceed Guidance values (reference and HBM values), where they are available? What data gaps exist?
3. Are there geographical differences and differences caused by industrial sector in the exposure of workers in EU to reprotoxic aprotic solvents?
4. What is the current exposure of the general EU population to reprotoxic aprotic solvents, especially with respect to females at reproductive age as well as mothers and their young children, and do they exceed Guidance values (reference and HBM values), where they are available? What data gaps exist?
5. What are the environmental concentrations of reprotoxic aprotic solvents in different environmental media and what is their geographical distribution and time trend in EU, and can they contribute to the overall exposure of the general population? What data gaps exist?
6. What are the indoor air and dust concentrations of reprotoxic aprotic solvents?
7. What is the content of reprotoxic aprotic solvents in widely used commodities (cosmetics, washing & cleaning products, paints, textiles, leather, etc.)?
8. How the exposure of general population to reprotoxic aprotic solvents is correlated with lifestyle and consumption patterns, what is the main exposure route?
9. Are there differences in exposure of the general EU population to regulated and non-regulated reprotoxic aprotic solvents (banned use in cosmetics)?
10. Are there differences in exposure of the workers in EU in relation to regulated and non-regulated reprotoxic aprotic solvents after the restriction for NMP will enter into force after 9 May 2020?
11. What are differences in profiles of reprotoxic aprotic solvents observed in exposure assessment regarding occupational environment and in relation to general public taking into account spatial and temporal distribution?
12. What are the mixture effects of aprotic solvents as a whole in relation to human exposure and how it can be estimated?
13. What are the best indicator`s substances (markers) to identify hazardous exposures to aprotic solvents as a whole?
14. What are the analytical options available with respect to aprotic solvents (gas chromatography-mass spectrometry versus liquid chromatography-tandem mass spectrometry for biological matrices, other methods in addition, methods for environmental media)?
15. What are the levels of reprotoxic aprotic solvents and associated health effects in vulnerable population groups, namely, mothers and their young children?
16. Are there other potentially hazardous aprotic solvents apart from the four reprotoxic aprotic solvents in question?
17. What is the state - of - the – art regarding chemical safety`s legislation on reprotoxic aprotic solvents in question and other potentially hazardous aprotic solvents identified?
18. Can reference values be established for any reprotoxic aprotic solvent in the case they are missing?
19. Can biomarkers of health effects be developed?

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7.4 Research Activities to be undertaken

Table 7-3: Listing of research activities to be carried out to answer the policy questions summed up in 4

Policy question	Substance	Available knowledge	Knowledge gaps and activities needed
1, 2, 3, 4, 5, 6, 7, 8, 10	NMP	<p>Toxicological information.</p> <p>Established biomarkers of exposure and HBM values.</p> <p>Analytical methods in place.</p> <p>Notion on the most significant exposure route. Some information on external and internal exposure in the occupational environment.</p>	<p>Very general knowledge about releases to environment – the related information should be gathered.</p> <p>No information on contamination of different environmental media – published information must be searched and environmental monitoring should be arranged in different geographical locations within EU.</p> <p>No information on content in widely used consumers' products – investigations should be arranged.</p> <p>Information on indoor pollution is lacking – special investigations should be arranged.</p> <p>Lacking information on exposure in the general population - published information must be searched and biomonitoring shall be arranged, especially in relation to vulnerable population groups, namely, females at reproductive age, mothers and their young children. Spatial (geographical) and temporal distribution shall be followed-up.</p> <p>No systematic investigations on exposure levels caused by different industrial sectors and geographical locations within EU – such information should be gathered by additional literature search.</p> <p>Information on REACH restriction success is lacking – such investigations shall be done after the transitional period.</p> <p>Association between exposure of general population and lifestyle and consumption patterns is unclear – special investigations shall be arranged.</p>

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Policy question	Substance	Available knowledge	Knowledge gaps and activities needed
1, 2, 3, 4, 5, 6, 7, 8, 9	DMF	Toxicological information. Established biomarkers of exposure and HBM values. Analytical methods in place. Notion on the most significant exposure route. Some information on external and internal exposure in the occupational environment.	<p>Very general knowledge about releases to environment – the related information should be gathered.</p> <p>No information on contamination of different environmental media – published information must be searched and environmental monitoring should be arranged in different geographical locations within EU.</p> <p>No information on content in widely used consumers` products – investigations should be arranged.</p> <p>Information on indoor pollution is lacking – special investigations should be arranged.</p> <p>Lacking information on exposure in the general population - published information must be searched and biomonitoring shall be arranged, especially in relation to vulnerable population groups, namely, females at reproductive age, mothers and their young children. Spatial (geographical) and temporal distribution shall be followed-up.</p> <p>No systematic investigations on exposure levels caused by different industrial sectors and geographical locations within EU – such information should be gathered by additional literature search.</p> <p>Information on success in relation to prohibition in cosmetic products is unclear - such investigations shall be done.</p> <p>Association between exposure of general population and lifestyle and consumption patterns is unclear – special investigations shall be arranged.</p>

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Policy question	Substance	Available knowledge	Knowledge gaps and activities needed
1, 2, 3, 4, 5, 6, 7, 8, 9	DMAC	Toxicological information. Established biomarkers of exposure and HBM values. Analytical methods in place. Notion on the most significant exposure route. Limited information on external and internal exposure in the occupational environment.	<p>Very general knowledge about releases to environment – the related information should be gathered.</p> <p>No information on contamination of different environmental media – published information must be searched and environmental monitoring should be arranged in different geographical locations within EU.</p> <p>No information on content in widely used consumers` products – investigations should be arranged.</p> <p>Information on indoor pollution is lacking – special investigations should be arranged.</p> <p>Lacking information on exposure in the general population - published information must be searched and biomonitoring shall be arranged, especially in relation to vulnerable population groups, namely, females at reproductive age, mothers and their young children. Spatial (geographical) and temporal distribution shall be followed-up.</p> <p>No systematic investigations on exposure levels caused by different industrial sectors and geographical locations within EU – such information should be gathered by additional literature search.</p> <p>Information on success in relation to prohibition in cosmetic products is unclear - such investigations shall be done.</p> <p>Association between exposure of general population and lifestyle and consumption patterns is unclear – special investigations shall be arranged.</p>

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Policy question	Substance	Available knowledge	Knowledge gaps and activities needed
1, 2, 3, 4, 5, 6, 7, 8	NEP	<p>Toxicological information.</p> <p>Established biomarkers of exposure and HBM values.</p> <p>Analytical methods in place.</p> <p>Notion on the most significant exposure route.</p>	<p>Very general knowledge about releases to environment – the related information should be gathered.</p> <p>No information on contamination of different environmental media – published information must be searched and environmental monitoring should be arranged in different geographical locations within EU.</p> <p>No information on content in widely used consumers` products – investigations should be arranged.</p> <p>Information on indoor pollution is lacking – special investigations should be arranged.</p> <p>Lacking information on exposure in the general population and in the occupational environment - published information must be searched and biomonitoring shall be arranged, especially in relation to vulnerable population groups, namely, females at reproductive age, mothers and their young children. Spatial (geographical) and temporal distribution shall be followed-up.</p> <p>No systematic investigations on exposure levels caused by different industrial sectors and geographical locations within EU – such information should be gathered by additional literature search.</p> <p>Association between exposure of general population and lifestyle and consumption patterns is unclear – special investigations shall be arranged.</p>

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Policy question	Substance	Available knowledge	Knowledge gaps and activities needed
11, 12, 13, 14, 15, 17, 18, 19	NMP, DMF, DMAC, NEP	Toxicological information. Established biomarkers of exposure and some HBM values. Analytical methods in place. Restricted external and internal exposure information in the occupational environment is in place.	Differences in profiles of reprotoxic aprotic solvents in relation to exposure and mixture effect is unclear – special investigations shall be done, possibilities to come to one common indicator substance (biomarker) should be assessed. No knowledge on biomarkers of health effects – special investigations shall be arranged. Contradictory information on applicability of different analytical methods – available methods shall be assessed, possibility an necessity to develop new methods should be assessed, interlaboratory validation exercises shall be arranged. Association between exposure of vulnerable population groups and related health effects is unclear – special investigations shall be arranged. No reference values including HBM values for all reprotoxic aprotic solvents – the missing reference values shall be developed.
16, 17	Other aprotic solvents	-	Lacking knowledge on possible other hazardous aprotic solvents– additional screening should be done and potential other priority aprotic solvents should be identified, their legal status should be investigated.

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8 Prioritised substance group: Arsenic

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8.1 Background Information

Arsenic (As) is a significant global environmental toxicant. As contamination of soil and drinking water is a problem threatening human health all over the world. Humans are exposed to As through the intake of air, food and water, and occupational exposure occurs in several industries including gold mining and smelting operations. Arsenic is carcinogenic (Group 1 IARC), studies confirming the carcinogenesis of arsenic in humans are identified, but are not reviewed in detail. It is well established that chronic exposure to As is associated with skin, lung and bladder cancers (IARC 1984; 2012, 2014; Helene et al. 2007; Järup et al. 1989; Lauwerys et al. 2001) as well as vascular diseases and hepatotoxicity (NRC 2001). For the general population, the principal route of exposure to arsenic is likely to be the oral route, primarily in the food and in the drinking water. The daily intake of total arsenic from food and beverages is generally in the range of 20–300 mcg/day. Therefore, assessment of exposures from natural sources of inorganic arsenic from diet, water and air would be helpful for risk communication and public health decision-making. Recent attention has also been directed at children's exposure to arsenic and potential health risks, because children are the most vulnerable and sensitive group to the adverse effects of arsenic. Understanding how arsenic exposures from human activities compare to natural background exposures is important for communicating the relative magnitude of calculated risks in perspective with everyday exposures. A number of issues are still to be addressed in HBM for arsenic: selection of exposure biomarkers, the role of genetic polymorphisms in contributing to population variability in pharmacokinetics and sensitivity to the adverse effects of exposure to arsenic etc.

This scoping document focuses on environmental exposure to arsenic (inorganic), which poses the greatest risk for human health.

8.1.1 Hazardous properties

Arsenic (metallic As, CAS numer: 7440-38-2; EC number: 231-148-6). Arsenic is a ubiquitous element that ranks 20th in abundance in the earth's crust. [Mandal & Suzuki 2002]. Arsenic is classified as a metalloid. Elemental arsenic is a steel grey solid material. Arsenic in the environment is combined with other elements such as oxygen, chlorine, and sulfur, and is called as inorganic arsenic. Of the inorganic arsenic compounds, arsenic trioxide, sodium arsenite and arsenic trichloride are the most common trivalent compounds, and arsenic pentoxide, arsenic acid and arsenates (e.g. lead arsenate and calcium arsenate) are the most common pentavalent compounds. (WHO 2000, ASTDR 2007)

Common organic arsenic compounds include arsanilic acid, methylarsonic acid, dimethylarsinic acid (cacodylic acid), and arsenobetaine (WHO, 2000).

Most inorganic and organic arsenic compounds are white or colorless powders that do not evaporate. They have no smell, and most have no special taste. [ASTDR 2007]. Arsenic in its most recoverable form is found in various types of metalliferous deposits.

It is common in iron pyrite, galena, chalcopyrite and less common in sphalerite. The most common arsenic mineral is arsenopyrite [Mandal & Suzuki 2002].

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The primary use of arsenic is in alloys of lead. Arsenic is a common n-type dopant in semiconductor electronic devices, and the optoelectronic compound gallium arsenide is the second most commonly used semiconductor after doped silicon. Arsenic and its compounds, especially the trioxide, are used in the production of pesticides, treated wood products, herbicides, and insecticides. Although arsenic can be poisonous in higher doses, it has also been used in some medicines. A form of arsenic is still used to treat an uncommon blood cancer known as *acute promyelocytic leukemia*. [Grund et al. 2008]

According to the International Agency for Research on Cancer (IARC), arsenic is classified in Group 1 (*sufficient evidence of carcinogenicity* in humans) In contrast to organic arsenic, iAs is extremely toxic and current risk assessments of dietary exposure to arsenic are entirely based on the inorganic forms. The general population is exposed to iAs via the diet, with food being the major contributor to intake when arsenic concentrations in water are <10 µg/L (the WHO guideline value for drinking water), while drinking water becomes the major source of exposure to iAs when water with arsenic concentrations well above 10 µg/L is used for drinking and cooking (EFSA, 2014; FAO/WHO, 2011). The IARC has established a causal role for oral exposure to iAs on skin, lung, and bladder cancers, and has shown suggestive evidence for liver, kidney, and prostate cancers (IARC, 2012). Apart from cancer – and skin lesions (EFSA, 2014) – a wide range of other adverse health effects such as cardiovascular diseases, developmental toxicity, abnormal glucose metabolism, type II diabetes and neurotoxicity are likely related to chronic ingestion of iAs (FAO/WHO, 2011). Susceptibility to the toxic effects of iAs varies considerably between individuals and populations depending on variations in iAs metabolism related to such factors as age, gender, life stage (e.g. pregnancy, lactation), nutritional status, and genetic polymorphisms in the regulation of enzymes responsible for iAs biotransformation (EFSA, 2014).

8.1.1.1 Knowledge gaps

The assessment of occupational exposure to inorganic arsenic iAs or/and sum of inorganic As is relatively well known (Janasik et al. 2014, Appostoli and al. 1999, Hakala and Pyy 1995.). The effects of general population exposure mainly concern exposure to As with potable water with an As content above 50 µg/L and concern mainly non-European populations. There is little work on the assessment of exposure to drinking water with concentrations below the limit and dietary As intake for European general populations.

Key epidemiologic evidence for risk assessment of dietary iAs comes from populations chronically exposed to high arsenic levels in drinking water (>50 µg/L) in several countries, including southwestern Taiwan (Chen et al., 2010), Bangladesh (Kurokawa et al., 2001), northern Chile (Smith et al., 1998), and Argentina (Hopenhayn-Rich et al., 1998.). The main source of As in the diet is organic As compounds such as arsenobetaine, which, is generally assumed to be of no toxicological concern (FAO/WHO, 2011). Dimethylarsinic acid (DMA) – and in traces monomethylarsinic acid (MMA) – are present in various foods, including rice, other plant-derived food and seafood. In vivo studies have shown adverse effects on the urinary bladder, kidneys, thyroid, and foetal development for DMA, whereas the gastrointestinal tract is the primary target organ of MMA (US FDA, 2016). The studies in animals showed a carcinogenic potential for DMA; however the data regarding human carcinogenicity are inconclusive, hence IARC classified these methylated forms as possibly carcinogenic to humans (Group 2B) (IARC, 2012).

Arsenosugars and arsenolipids are mainly metabolised in humans to DMA, and limited albeit growing information is available regarding their toxicity (Taylor et al., this issue).

Along with MMA and DMA, these compounds have been proposed to be classified as 'potentially toxic' from a food safety perspective, in contrast to the innocuous arsenobetaine (Feldmann and Krupp, 2011). (Quoted by Cubadda et al 2017).

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As a result, exposure levels for iAs with no appreciable health risk, i.e. a tolerable daily or weekly intake, cannot be identified. Instead, reference points for health protection are currently based on benchmark responses of a given percentage of extra risk from human data. A benchmark dose lower confidence limit (BMDL) for 0.5% excess risk of lung cancer has been established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (BMDL0.5 = 3 µg/kg bw/day) (FAO/WHO, 2011), whereas the European Food Safety Authority (EFSA) identified a range of BMDL values for 1% excess risk of cancers of the lung, skin and bladder, as well as skin lesions (BMDL01 = 0.3-8 µg/kg bw/day) (EFSA, 2009). Therefore, for risk characterisation an assessment of the margins of continues to emerge exposure (MOEs) between the identified reference points and the estimated daily dietary exposure to iAs is required, since there are no exposure levels associated with the absence of appreciable health risk on long-term (lifetime) basis (Quoted by Cubadda et al 2017).

EFSA and JECFA data assessments are relatively recent, new scientific evidence of adverse effects for populations chronically exposed to iAs via drinking water in concentrations below 50 µg/L, are discussed by other authors (D'Ippoliti et al., 2015; Leonardi et al., 2012; Garcia-Esquinas et al., 2013; Moon et al., 2013; Zheng et al., 2013). Such evidence has not fed into a new risk assessment yet.

The category proposed for arsenic and its inorganic compounds is Category B, as HBM data for arsenic as a food and drinking water contaminant are available, but at insufficient level to provide an overall picture of exposure in Europe. Identified data gaps may vary from spatial gaps in HBM measurement data, to gaps in exposure sources and pathways. Inorganic arsenic is regulated as to drinking water and OELs. There is a toxicological concern because of carcinogenicity and suggested reproductive and neurodevelopmental toxicity of arsenic, as well as the low dose effects that relate to cardiovascular diseases, insulin resistance, type-2 diabetes and hypertension. [NRC 2014; Nachman et al 2017; Navas-Acien et al 2005, 2006; Abhyankar et al 2012].

8.1.2 Exposure characteristics

8.1.2.1 Environmental behaviour

Arsenic is found in the environment in the metallic form and in various inorganic and organic complexes. The sources are both natural and anthropogenic.

Soil: Arsenic occurs naturally in soils as a result of the weathering of the parent rock.

Anthropogenic activity has resulted in the widespread atmospheric deposition of arsenic the burning of coal and the smelting of non-ferrous metals including copper [EPA 2009a]. The levels of arsenic in the soils of various countries are said to range from 0.1 to 40 mg/kg (mean 6 mg/kg), 1 to 50 mg/kg (mean 6 mg/kg) and mean 5 mg/kg but varies considerably among geographic regions.

Arsenic is present in soils in higher concentrations than those in rocks [Mandal & Suzuki 2002]. Uncontaminated soils usually contain 1–40 mg/kg of arsenic, with lowest concentrations in sandy soils and those derived from granites, whereas larger concentrations are found in alluvial and organic soils. Arsenate reportedly binds strongly to iron and manganese oxides, and therefore remains in the surface soil layer after deposition [ATSDR, 2007]. Arsenic was observed to be still concentrated after 15 years in the top 20–40 cm of orchard soils treated with lead arsenate (Merwin et al. 1994). However, several experimental studies have found that arsenate can be released from iron oxides at alkaline pH as a result of desorption processes [IPCS, 2001; ATSDR, 2007].

Water: Arsenic is found at low concentration in natural water. The maximum permissible concentration of arsenic in drinking water is 50 mcg/l and recommended value is 10 mcg/l by EPA

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and WHO [IPCS 2001]. The seawater ordinarily contains 0.001–0.008 mg/l of arsenic. The concentration of arsenic in unpolluted fresh waters typically ranges from 1–10 g/l, rising to 100–5000 g/l in areas of sulfide mineralisation and mining [Mandal & Suzuki 2002].

Only a very minor fraction of the total arsenic in the oceans remains in solution in seawater, as the majority is sorbed on to suspended particulate materials. The high levels of arsenic are in waters from areas of thermal activity in New Zealand up to 8.5 mg/l. Geothermal water in Japan contains 1.8–6.4 mg/l and neighboring streams about 0.002 mg/l. Although normally groundwater does not contain methylated form of arsenic but lake and pond waters contain arsenite, arsenate as well as methylated forms, i.e. MMA and DMA [Mandal & Suzuki 2002].

Air: In air, arsenic exists predominantly adsorbed on particulate matters, and is usually present as a mixture of arsenite and arsenate, with the organic species being of negligible importance except in areas of arsenic pesticide application or biotic activity [Mandal & Suzuki 2002]. The human exposure of arsenic through air is generally very low and normally arsenic concentrations in air ranges from 0.4 to 30 ng/m³. According to USEPA the estimated average national exposure in the U.S. is at 6 ng As/m³. Absorption of inhaled arsenic ranges between 30 and 85%, depending on the relative portions of vapour and particulate matters. USEPA estimates that the general public will be exposed to a range of approximately 40–90 ng per day by inhalation. The amount of arsenic inhaled per day is about 50 ng or less (assuming that about 20 m³ of air is inhaled per day) in unpolluted areas. The daily respiratory intake of arsenic is approximately 120 ng of which 30 ng would be adsorbed. Typical arsenic levels for the European region are currently quoted as being between 0.2 and 1.5 ng/m³ in rural areas, 0.5 and 3 ng/m³ in urban areas and no more than 50 ng/m³ in industrial areas. [European Commission 2000]

Animals and human beings: As in plant tissue, arsenic is cumulative in animal tissue, allowing for a wide variation in concentration due to the variance in arsenic ingested in different areas. Among marine animals, arsenic is found to be accumulative to levels of from 0.005 to 0.3 mg/kg in coelenterates, some molluscs and crustaceans. Some shellfish may contain over 100 mcg/g of arsenic. The average arsenic content in freshwater fish is of 0.54 mcg/g on the basis of total wet weight, but some values reach as high as 77.0 mcg/g in the liver oil of freshwater bass. In mammals it is found that the arsenic accumulates in certain areas of the ectodermic tissue, primarily the hair and nails [Mandal & Suzuki 2002].

Human exposure: Humans are exposed to many different forms of inorganic and organic arsenic species (arsenicals) in food, water and other environmental media. Each of the forms of arsenic has different physicochemical properties and bioavailability and therefore the study of the kinetics and metabolism of arsenicals is a complex matter.

General population: For the general population, the principal route of exposure to arsenic is likely to be the oral route, primarily via food, and drinking water. Intake from air, is usually much less. Dermal exposure can occur, but is not considered a primary route of exposure. The epidemiologic evidence for an across the placenta is insufficient, although there exists limited evidence for arsenic concentrations found in cord blood and maternal blood of maternal-infant pairs exposed to high arsenic-containing drinking water. [ASTDR 2007.]

Occupational exposure population: Occupational exposure to arsenic may be significant in several industries, mainly nonferrous smelting, arsenic production, wood preservation, glass manufacturing. Occupational exposure would be via inhalation and dermal contact.

Human Biomonitoring (HBM)

HBM can be defined as “the method for assessing human exposure to chemicals or their effects by measuring these chemicals, their metabolites or reaction products in human specimens [CDC,

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2005]. Biomonitoring data directly reflect the total body burden or biological effect resulting from all routes of exposure, and interindividual variability in exposure levels, metabolism and excretion rates.

The cytotoxicity and metabolism of arsenic is a function of its oxidation state and methylation status [Cohen et al. 2007]. Metabolic conversion of inorganic arsenic into methylated products is a multistep process that yields mono-, di-, and trimethylated arsenicals. In recent years, it has become apparent that formation of methylated metabolites of inorganic arsenic is not necessarily a detoxification process. Products formed in this pathway may be more reactive and toxic than inorganic arsenic [Thomas et al. 2007]. Inorganic arsenic are commonly methylated in liver in the presence of a methyl donor S-adenosylmethionine (SAM) and a co-factor glutathione (GSH) with arsenomethyltransferase (As3MT) to relevant monomethylated [e.g., monomethylarsonous acid (MMA^{III}) monomethylarsonic acid (MMA^V)] and dimethylated arsenic metabolites [e.g., dimethylarsinous acid (DMA^{III}), dimethylarsinic acid (DMA^V)], and finally excreted into urine [Vahter et al. 1999; Vahter 2002]. Recently, a reductive methylation pathway has also been described [Tseng 2009]. Following arsenic exposure, 40 to 60% of arsenic intake is eliminated through urine. It should also be mentioned that the majority of the environmentally exposed population groups studied so far have on average 10-30% of inorganic As, 10-20% of MMA and 60-70% of DMA in urine, but considerable inter-individual variations have been observed, which may be a result of genetic polymorphism in the methylation capacity of arsenic (Vahter 1999).

Urinary levels of arsenic are generally regarded as a good measure and biomarker of exposure, although measurements of total arsenic in urine do not contain information concerning arsenic species, thereby complicating the assignment of toxicity and potential health risk to various species of As. Quantitative determination of the amount of a specific element is particularly important and that is why speciation methods are considered essential for drawing accurate conclusions in arsenic exposure and risk assessment.

For many years, biological monitoring of exposure to arsenic has been based on the determination of the sum of iAs and methylated metabolites DMA and MMA in urine. Novel biomonitoring methods (speciation analysis) are usually tested and validated in research settings (Janasik et al. 2014). Sustained national and international surveillance programmes typically use well established biomonitoring techniques (e.g. biomarkers which are known to reflect exposure to the chemical of interest, standardised sampling methods and verified analytical techniques) to collect information on population exposures to environmental hazards that are known to be significant to public health.

A summary of available Human Biomonitoring from EU countries on arsenic exposure are summarised in a report from the World Health Organization (2015) and are shown in the table below.

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Table 8-1: Summary of available HBM data on arsenic (toxicologically relevant species including inorganic arsenic and its metabolites) Geometric means (GM) or percentiles (P90/P95) are indicated

Country	Study	Population (N)	Total arsenic			TRA species	references
			Blood (ng/mL)	Urine (µg/g creat.)	Urine (µg/L)	Urine (µg/g creat.)	
Belgium (Flanders)	FLESH (2007-2011)	Neonates (241)	0.54 GM 2.18 P90	-	-	-	Schoeters et al., 2012a
		Mothers Age: 20-40 y (235)	0.64 GM 2.04 P90	15.9 GM 71.4 P90	-	3.7 GM 10.7 P90	
		Adolescents Age: 14-15 y (207)	0.62 GM 2.12 P90	9.3 GM 49.0 P90	-	3.6 GM 8.0 P90	
Germany	Environmental Specimen Bank (2000-2017), four sampling locations	Young adults Age: 20-29 y		4.4- 5.5 GM			www.umweltprobenbank.de (2017)
	GerES I (1985-86)	Adults Age: 25-69 y (2542)	-	-	9.02 GM 37.5 P95	-	Kolossa-Gehring et al., 2012; Schulz et al., 2007b
	GerES II (1990-92)	Adults Age: 18-79 y (4001)	0.5 GM 2.0 P95	-	6.33 GM 30.2 P95	-	
		Children Age: 6-17 y (731)	0.33 GM 1.4 P95	-	6.01 GM 27.5 P95	-	
	GerES III (1998)	Adults Age: 18-69 y (4052)	0.61 GM 2.4 P95	-	3.87 GM 19.3 P95	-	
	GerES IV (2003-2006)	Children Age: 3-14 y (1734)	0.23 GM 0.3 P90	-	4.4 GM 11.0 P90	-	
France	ENNS (2006-2007)	Adults 18-74 y (1515)	-	11.96 GM 61.29 P95	-	3.34 GM 8.9 P95	Frery et al., 2012
Italy	PROBE (2008-2010)	Adolescents Age: 13-15 y (252)	0.82 GM 3.69 P95	-	-	-	Pino et al., 2012
Slovenia	National HBM Survey (2007-2009)	Adults Age: 20-40 y (274)	0.74 GM 2.98 P95	-	-	-	Snoj Tratnik, Mazej & Horvat, 2012

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8.1.2.2 Health based guidance values available for HBM data

General population

The following table summarises the available reference values for Canadian and German population.

Table 8-2: Reference values (RV95) for arsenic in blood and urine based on Human Biomonitoring data

	Population	Group (years)	Years (N)	P95 (95% CI) (µg/L)	RV95 (µg/L)	References
arsenic (total) in blood	Canadian	6-19	2007-2009 (875)	1.4 (1.0-1.8)	1.4	Saravanabhavan et al. (2017)
arsenic (total) in blood	Canadian	20-79	2007-2009 (996)	2.0 (1.8-2.2)	2.0	
Arsenic (total) in urine	German	3-14*	2003-2006		15.0	Schultz et al.(2011)
Arsenic (total) in urine	German	18-69*	1997-1999		15.0	

* for children and adults who did not eat fish during 48 hours prior to sample collection

The RV 95 for total arsenic in urine, according to the findings of the German HBM survey, is 15 µg/L for children and adults who did not eat fish during 48 hours prior to sample collection [Schulz et al., 2011]. The GM levels of total arsenic in European populations were from 0.5 µg/L to 1 µg/L in blood and from 4µg/g to 16 µg/g creatinine in urine. There was no obvious difference observed between children/adolescents and adults.[WHO 2015]

In order to establish representative Human Biomonitoring data for the Canadian general population, an extensive HBM component has been incorporated into the Canadian Health Measures Survey (CHMS). The CHMS, which was launched in 2007, is the most comprehensive direct health measures survey conducted in Canada and is designed to provide nationally-representative data on indicators of environmental exposures, health and nutritional status, and related risks and protective characteristics [Tremblay et al., 2007].

Occupational population

The following recommendations are available:

Table 8-3: Recommended Biological Limit Values (BLV) for occupational exposure

Organisation	Biological Limit Value (BLV)	Reference
Germany/ Deutsche Forschungsgemeinschaft (DFG)	<ul style="list-style-type: none"> Inorganic arsenic and methylated metabolites BLW 50 mcg/l Arsenic(+III) BAR 0.5 mcg/l Arsenic(+V) BAR 0.5 mcg/l Monomethylarsonic acid BAR 2 mcg/l Dimethylarsinic acid BAR 10 mg/l 	DFG 2016
USA/ ACGIH	<ul style="list-style-type: none"> 35 mcg arsenic/L of urine (inorganic arsenic plus methylated metabolites) 	ACGIH

BAR ("Biologische Arbeitsstoff-Referenzwerte") describe the background level of a substance which is present concurrently at a particular time in a reference population of persons of working age who are not occupationally exposed to this substance. The BAR are based on the 95th percentile without regarding effects on health.

BLW ("Biologischer Leit-Wert") is the amount of a chemical substance or its metabolites or the deviation from the norm of biological parameters induced by the substance in exposed humans which serves as an indicator for necessary protective measures. BLWs are assigned only for hazardous materials for which the available toxicological or occupational-medical data are insufficient for the establishment of BAT values[DFG 2016]

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8.1.3 Policy relevance

8.1.3.1 European Policies

European legislations concerning arsenic are described below.

8.1.3.2 Food safety

Maximum levels for arsenic in certain foods have been established by [Commission Regulation \(EC\) No 2015/1006](#) (future section 3.5 of the Annex to Regulation (EC) No 2006/1881, applicable from 1 January 2016 onwards).

The EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) assessed the risks to human health related to the presence of arsenic in food. More than 100,000 occurrence data on arsenic in food were considered with approximately 98 % reported as total arsenic. Making a number of assumptions for the contribution of inorganic arsenic to total arsenic, the inorganic arsenic exposure from food and water across 19 European countries, using lower bound and upper bound concentrations, has been estimated to range from 0.13 to 0.56 µg/kg bodyweight (b.w.) per day for average consumers, and from 0.37 to 1.22 µg/kg b.w. per day for 95th percentile consumers. Dietary exposure to inorganic arsenic for children under three years of age is in general estimated to be from 2 to 3-fold higher that of adults. The CONTAM Panel concluded that the provisional tolerable weekly intake (PTWI) of 15 µg/kg b.w. established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) is no longer appropriate as data had shown that inorganic arsenic causes cancer of the lung and urinary bladder in addition to skin, and that a range of adverse effects had been reported at exposures lower than those reviewed by the JECFA. The CONTAM Panel modelled the dose-response data from key epidemiological studies and selected a benchmark response of 1 % extra risk of cancers of the lung, skin and bladder, as well as skin lesions (BMDL₀₁ = 0.3-8 µg/kg bw/day).

The estimated dietary exposures to inorganic arsenic for average and high level consumers in Europe are within the range of the BMDL01 values identified, and therefore there is little or no margin of exposure and the possibility of a risk to some consumers cannot be excluded.

8.1.3.3 Chemicals

Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006, concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals, Official Journal No. L 396/1 of 30.12.2006 (hereinafter "REACH") aims at ensuring a high level of protection for human health and environment, while promoting the efficient functioning of the EU internal market and stimulating innovation and competitiveness in the chemical industry.

Having a common interest in fulfilling the requirements under REACH, the members of the As Consortium have therefore created the As Consortium back in 2009, in order to share human and financial resources involved in complying with REACH.

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The following substances were REACH registered with the help of the As consortium:

Name	Molecular formula	EC	CAS	Registered	No of registrants	LR	Authorisation
Arsenic metal	As	231-148-6	7440-38-2	yes		ppm	
Arsenic trichloride	AsCl ₃	232-059-5	7784-34-1	yes		ppm	
Diarsenic trioxide	As ₂ O ₃	215-481-4	1327-53-3	yes	6	umicore	Boliden/nordenhamer/ zinhutte/linxens Fr
Gallium arsenide	GaAs	215-114-8	1303-00-0	yes	3	FCM	
Trilead diarsenate	Pb ₃ (AsO ₄) ₂	222-979-5	3687-31-8	Yes, INACTIVE	1		
Calcium arsenate	Ca ₃ (AsO ₄) ₂	231-904-5	7778-44-1	Yes, INACTIVE	1		
Tricopper arsenide	Cu ₃ As	234-472-6	12005-75-3	Yes, INACTIVE	1		

**The Arsenic consortium, was founded in 2009(members are producers and importers of arsenic and arsenic compounds). The Consortium Members controlle complying with the requirements of the REACH Regulation in respect of the substance(s) covered by the Consortium and to follow up on environment, health and safety (EHS) regulations related to arsenic and arsenic compounds. The consortium includes, among others: UMICORE (Belgium),AURUBIS (Germany) and Boliden Harjavalta Oy (Finland)*

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According to the harmonised classification and labelling (CLP) approved by the European Union, this substance is toxic if swallowed, is toxic if inhaled, is very toxic to aquatic life and is very toxic to aquatic life with long lasting effects. Moreover, some uses of this substance are restricted under Annex XVII of REACH.

Occupational health and safety

Proposal for a DIRECTIVE OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL amending Directive 2004/37/EC on the protection of workers from the risks related to exposure to carcinogens or mutagens at work. This proposal aims to improve workers' health protection by reducing occupational exposure to five carcinogenic chemical agents, to provide more clarity for workers, employers and enforcers, and to contribute to a level playing field for economic operators.

8.1.4 Technical aspects

8.1.4.1 Availability of biomarkers and methods

There are several potential biomarkers for arsenic exposures. Preferred biomarkers are determination of As and its chemical forms in urine. Non-invasive, ease collection and because the majority of absorbed arsenic and its metabolites is eliminated via urine puts this type of markings in a privileged position. Moreover, the analytical techniques allows arsenic speciation in urine, but not hair and nails (due to mineralisation). The short half-life of inorganic and organic arsenic species in blood and invasive collection limits the utility of arsenic biomarkers in blood, similar to determination As in hair and nails. Advantages for these biomarkers in hair and nails are is assessment of integrated exposures, but these markers include arsenic derived from all way organic arsenic (non-toxic) and inorganic species. (Hughes 2006; Navas-Acien and Guallar, 2008). When exposure to a compound results in multiple biomarkers and the mode of action is not known with certainty, it is recommended to sum as many of the metabolites in a Biomonitoring Equivalent (BE) calculation as long as the metabolites are specific to exposures of concern (Aylward et al., 2009). Sum of iAs, MMA, DMA correlate well with drinking water concentration (Calderon et al., 1999; Hall et al., 2006) or estimated daily dose calculated using drinking water concentrations (Navas-Acien et al., 2009; Agusa et al., 2009). The concentrations of total arsenic and iAs, MMA, and DMA are all fairly constant over time with small intra-individual variabilities (Navas-Acien et al., 2009; Kile et al., 2009). First morning voids of total arsenic are indicative of and correlated with subsequent voids throughout the day (Calderon et al., 1999). For these reasons, speciated arsenic in urine (iAs III, iAs V, MMA, and DMA) are the preferred biomarker(s) for exposures to inorganic arsenic (Lauwerys and Hoet, 2001) but as described Buchet et al., 1994 certain types of seafood can contain small quantities of DMA than the urine sample should abstain from eating seafood for 3–4 days prior to urine collection (Lauwerys and Hoet, 2001). In such cases where diet cannot be controlled, Lauwerys and Hoet (2001) have recommended using iAs concentration in urine as opposed to the sum of iAs, MMA, and DMA in urine as the biomarker of choice. Since MMA is not affected by seafood consumption, both iAs and MMA should be reliable biomarkers of inorganic arsenic exposures. Then, the recommendations are for using sums of all three (iAs, MMA, and DMA) as a biomarkers for As .when no exposures to seafood have occurred.

The determination of arsenic in biological specimens requires sensitive analytical methods, performed under good quality control conditions. Various methods exist that differ in sample preparation technique and/or the detections system. Determination of total As concentration can be done by ICP MS, inorganic arsenic as well as MMA and DMA can be done by AAS technique with hydrogen generation.

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Speciation of arsenic requires coupled analytical techniques (ICP-MS-HPLC) and procedures and expensive reagents and equipment, which are not routinely available in analytical laboratories. Speciation analysis is necessary to differentiate between inorganic and organic arsenic exposure.

Need for new approaches

The symptoms and signs caused by long-term elevated exposure to inorganic arsenic differ between individuals, population groups and geographical areas. Thus, there is no universal definition of the disease caused by arsenic. This complicates the assessment of the burden on health of arsenic.

There is a need to harmonise exposure biomarkers and to validate biomarkers of susceptibility, selection of exposure biomarkers, and include the role of genetic polymorphisms in contributing to population variability in pharmacokinetics and sensitivity to the adverse effects of exposure to arsenic.[Ladeira C, Viegas S. 2016; Chen et al. 2005; Janasik et al. 2018]

It is important to harmonise the approaches used to investigate different study populations. The selection of best suited matrices and biomarkers of exposure is crucial. Markers of susceptibility need to be validated. These are important for understanding the human health effects of low-level As exposure as a basis for future research efforts, risk assessment, and exposure remediation policies worldwide. As speciation in urine, would provide characterisation of species-specific exposure at levels relevant for European population. In recent years interest in gene-environment interaction has grown substantially, because of the progress in laboratory techniques, improved understanding of genetics and realisation of complex mechanisms between genetics and environment. Identification and validation of novel biomarkers of susceptibility is therefore an important part in investigation of exposure-health relationships.

8.1.5 Societal concern

Arsenic is one of WHO's 10 chemicals of major public health concern. The effects of arsenic toxicity on mental health and associated social consequences have not been well reported and hence more scientific attention is needed.

Arsenic contamination of groundwater is widespread and there are a number of regions where arsenic contamination of drinking-water is significant. It is now recognised that at least 140 million people in 50 countries have been drinking water containing arsenic at levels above the WHO provisional guideline value of 10 µg/L .

In 2010, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) re-evaluated the effects of arsenic on human health, taking new data into account. JECFA concluded that for certain regions of the world where concentrations of inorganic arsenic in drinking-water exceed 50–100 mcg/L, there is some evidence of adverse effects. In other areas, where arsenic concentrations in water are elevated (10–50 µg/L), JECFA concluded that while there is a possibility of adverse effects, these would be at a low incidence that would be difficult to detect in epidemiological studies.

The most important action in affected communities is the prevention of further exposure to arsenic by the provision of a safe water supply for drinking, food preparation and irrigation of food crops.

WHO's work to reduce arsenic exposure includes setting guideline values, reviewing evidence, and providing risk management recommendations. WHO publishes a guideline value for arsenic in its *Guidelines for drinking-water quality*. The Guidelines are intended for use as the basis for regulation and standard setting worldwide.

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The WHO/UNICEF Joint Monitoring Programme for Water Supply, Sanitation and Hygiene monitors progress towards global targets on drinking water. Under the new 2030 Agenda for Sustainable Development, the indicator of “safely managed drinking water services” calls for tracking the population accessing drinking water which is free of faecal contamination and priority chemical contaminants, including arsenic.

Due to its classification as a substance toxic to reproduction (“CRM” according to Annex VI of Regulation 1272/2008) arsenic is included in the “SIN (Substitute It Now!) List”, a comprehensive database of chemicals likely to be restricted or banned in the EU developed by the non-governmental European “International Chemical Secretariat” (ChemSec).

Arsenic ranks 1st out of 275, on the “Substance Priority List” (SPL) prepared biannually by the ATSDR for substances most commonly found at facilities on the National Priorities List (NPL) and which are determined to pose the most significant potential threat to human health due to their known or suspected toxicity and potential for human exposure. It should be noted that this priority list is not a list of “most toxic” substances, but rather a prioritisation of substances based on a combination of their frequency, toxicity, and potential for human exposure at NPL (national priority list) sites.

In June 2017, members of the Stakeholder Forum provided feedback on the proposed strategy and criteria to be used for the prioritisation of substances for monitoring and research under HBM4EU. Arsenic was voted by stakeholders who participated in the Stakeholder Workshop organised in the frame of HBM4EU in on November 20th 2017 as a “top substance of concern”.

8.2 Categorisation of Substances

The proposed category for Arsenic is Category B.

The category proposed for arsenic and its inorganic compounds is **Category B**, as HBM data for arsenic as a food and drinking water contaminant are available, but at insufficient level to provide an overall picture of exposure in Europe. Identified data gaps may vary from spatial gaps in HBM measurement data, to gaps in exposure sources and pathways. Inorganic arsenic is regulated as to drinking water and OELs. There is a toxicological concern because of carcinogenicity and suggested reproductive and neurodevelopmental toxicity of arsenic, as well as the low dose effects that relate to cardiovascular diseases, insulin resistance, type-2 diabetes and hypertension. The effects of chronic exposure to low levels and the factors of susceptibility have not been adequately investigated.

Table 8-4: Substances included in the substance group, listed according to availability of toxicology and human biomarker data, in category A, B, C,D,E substances

Category	Abbreviation/ Acronym	Systematic name	CAS No.	Regulations
B	As	Arsenic	7440-38-2	Regulation (EC) No 1907/2006 REACH Regulation (EC) No 1907/2006 for inclusion of substances in the Authorisation List (Annex XIV)

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8.3 Policy-related questions

The following policy-related questions relate to commitments under this frame.

1. What is the current exposure of the EU population to arsenic?
2. What biomonitoring and exposure (environmental and occupational) data on arsenic, relevant to the European population, are currently available?
3. What is the geographic spread of the current exposure and how does it relate to different exposure sources (environmental; dietary sources)?
4. Which population groups are most at risk?
5. What factors (genetic polymorphisms) make people more susceptible or not to the risk of health effects due to arsenic exposure? How are the best and more sensitive biomarkers for identification of reliable arsenic exposure and to link to potential adverse health-effect?
6. What are possible health effects resulting from chronic low exposure to arsenic from food consumption?
7. What are the best analytical methods should allow for differentiating species in urine?
8. How can harmonised, validated and comparable information be collected to support and evaluate current policies?
9. How can transfer of knowledge & technology be facilitated to support current policies?
10. How can HBM4EU results support European policy decisions?

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8.4 Research Activities to be undertaken

Table 8-5: Listing of research activities to be carried out to answer the policy questions

Policy question	Substance	Available knowledge	Knowledge gaps and activities needed
What is the current exposure of the EU population to arsenic?	As	Human exposure and effects data are limited.	Mapping and / or updating existing biomonitoring / exposure data <ul style="list-style-type: none"> ▶ collection, comparison, evaluation and integration into IPChem ▶ identification of knowledge gaps ▶ prioritisation of research needs WP 7/8/9/10
What biomonitoring and exposure (environmental and occupational) data on arsenic, relevant to the European population, are currently available.	As	Publications on occupational exposure are available, but the data is rather old and some exposures are not relevant anymore. Publications on environmental exposure are available, but the data is rather not EU population exposures and not included dietary sources (excluded water)	Mapping / updating existing toxicological/biomonitoring data collection, comparison, evaluation and integration into IPChem identification of knowledge gaps WP 7/8/9/10
What is the geographic spread of the current exposure and how does it relate to different exposure sources (environmental; dietary sources)?	As	Human exposure and effects data are limited.	Mapping of existing data on arsenic content in food and water including geographical variations in Europe. The term daily intake of arsenic depending on the geographic region and dietary habit. Use of existing data to assess the determinants of exposure, including geographic variations and their causes (e.g. environmental exposures, diet) identification of knowledge gaps
Which population groups are most at risk?	As	Studies in vulnerable populations and studies for a better understanding of the health effects of inorganic arsenic in the population at exposure levels in EU are greatly needed.	Establish European arsenic biomonitoring program covering broad population groups (children and adults).
What factors (genetic polymorphisms) make people more susceptible or not to the risk of health effects due to arsenic exposure? How is the best and more sensitive biomarkers for identification of reliable arsenic exposure and to link to potential adverse health-effect?	As	Human exposure and effects data are limited. Publications on influence of genetic polymorphisms on arsenic metabolism are available, but the data is rather not EU population exposures.	Mapping of existing capacities <ul style="list-style-type: none"> ▶ Explore the possible use of existing cohorts for the investigation of the adverse health effects due to chronic exposure to low levels of arsenic including the identification and possibly validation of markers of susceptibility ▶ Identification of reliable biomarkers (biochemical and/or molecular biology markers) of arsenic exposure and to link to potential adverse health-effect

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Policy question	Substance	Available knowledge	Knowledge gaps and activities needed
What are possible health effects resulting from chronic low exposure to arsenic from food consumption?	As	Human exposure and effects data are limited.	<ul style="list-style-type: none"> ▶ Identification of groups at risk of exceeding health-based guidance values, based on existing information (e.g. by age, gender, diet, geography, co-exposures, hot-spots in Europe) ▶ To determine whether current or expected exposure levels of As are of concern for health in the general population.
What is the safe intake level for arsenic that is without any appreciable health risk in the general European population?	As	The EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) assessed the risks to human health related to the presence of arsenic in food, but human exposure and effects data are limited.	<p>Preparation of a core study to assess:</p> <ul style="list-style-type: none"> ▶ (a) the current exposure of Europeans to arsenic and the associated risk and to facilitate the assessment of temporal trends with regards to the effectiveness of policies (b) the contributions of different sources (dietary, environmental,) to the body burden, with the aim to elaborate HBM threshold levels for Europe and safe upper limits for different types of foodstuff
What are the best analytical methods should allow for differentiating species in urine?	As	Recently developed HBM analytical methods should allow for differentiating species in urine, resulting from inorganic arsenic exposure, including As III, As V and twomethylated metabolic products, DMA and MMA.	<p>Mapping of existing capacities</p> <ul style="list-style-type: none"> ▶ cost-effective, reliable analytical methods capable of speciation analysis ▶ standard procedures for quality-controlled sampling ▶ qualified laboratories for sample analysis as result of the QA / QC program established in HBM4EU ▶ Arsenic different chemical form of should be included (speciation analysis). Laboratories that will apply for the determination of arsenic in biological material should be verified preceded by participation in the QA / QC program established by HBM4EU. ▶ Establishment of unified methods of biological material collection, storage and shipping procedures to centers, which will determine arsenic concentrations.
How can harmonised, validated and comparable information be collected to support and evaluate current policies?	As		Preparation of an inventory of current relevant national strategies in European countries

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Policy question	Substance	Available knowledge	Knowledge gaps and activities needed
How can HBM4EU results support European policy decisions?	As		<ul style="list-style-type: none"> ▶ Identification of stakeholders ▶ Mapping, prioritising and addressing stakeholder needs, starting with policy makers and scientists ▶ Describe previous studies identifying the impact of EU legislation ▶ Establish permanent European arsenic biomonitoring as support of arsenic european policies

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9 Prioritised substance group: Bisphenols

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9.1 Background Information

9.1.1 Hazardous properties

Bisphenol A

There is a large amount of literature on the toxicity of bisphenol A including at low doses [reviewed in WHO and UNEP (2012), Gore et al. (2015), Vandenberg (2014), and EFSA Journal (2015)]. Studies have indicated that it could be associated with increased risk for:

- ▶ Fetal development: miscarriages, decreased birth weight at term,
- ▶ Reproductive and sexual dysfunctions,
- ▶ Breast and prostate cancer or at least significant breast tissue remodelling. Studies have indicated that those effects were associated with gestational and neonatal exposure [Seachrist et al. (2016)].
- ▶ Altered immune system activity,
- ▶ Obesity and metabolic dysfunctions and diabetes in adults,
- ▶ Cardiovascular disease in adults
- ▶ Cognitive and behavioural development in young children.

Despite the wealth of studies, there are still controversies concerning the toxic effects of BPA. Those are related to some lack of reproducibility of the experimental studies possibly due to different designs as well as on issues related to the analytical procedures used for BPA assays. Several studies (both experimental and human) have focused on perinatal exposure using different doses including low doses and monitoring a variety of outcomes [FitzGerald and Wilks (2014)]. In human there are several cohort studies associating perinatal exposure and child development. In addition, there are cross-sectional studies where associations were found between BPA exposure and metabolic and cardiovascular diseases. The latter studies have established association but cannot reveal a causal link between BPA and a toxic outcome. In conclusion, there is a real concern that BPA exposure could be linked to a variety of health outcomes in human, with different level of evidence depending on the outcome and the exposure period. Other Bisphenols, notably many BPA substitutes structurally similar to BPA, have been less studied although data suggest they are also oestrogenic, and likely to induce similar health effects [Rochester and Bolden (2015), Shaleni et al. (2020)].

BPA elicits a variety of endocrine disrupting effects targeting steroid hormones as well as thyroid hormones. Several studies have explored the mechanisms of endocrine disruption. Initial studies have indicated an interaction with the nuclear ER alpha oestrogen receptor with a relatively low affinity. Further studies have indicated an interaction with other receptors such ERbeta,

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ERRgamma and GPR32. An unresolved question is which of those receptors is involved in the low dose fetal effects of BPA.

Because of the controversies on BPA toxicity, a collaborative project called CLARITY-BPA was carried out in the US involving both regulatory agencies and academic laboratories [National Toxicology Program Research report 9 (2018)]. Despite the fact that animal treatment was centralised, different outcomes and conclusions were reached by different groups. The core studies run by the FDA found little consistent evidence for toxicity and for non-monotonic dose-response curves when traditional outcomes were examined [Camacho et al. (2019)]. Studies done in academic laboratories found evidence for low-dose effects and non-monotonic dose-response [Prins et al. (2019)]. As an illustration, a recent scientific study, undertaken as part of the CLARITY-BPA project, developed a quantitative assessment of the effects of bisphenol A (BPA) exposure on mammary gland development and found a consistent pattern of non-monotonic dose response relationships on a set of over 90 measurements. This demonstrates a causal relationship between exposure to BPA and the health effects observed [Montévil et al. (2020)]. The reasons for these discrepancies are unclear and they could be related to a non-optimal study design, different health outcomes, different analysis of the data and different interpretation of some data.

Bisphenol S, F and others

Recent studies on BPS toxicity are published. Regarding BPS toxicity on reproduction in humans, maternal prenatal urinary BPS concentrations were consistently associated, but not significantly, with various markers of fetal growth [Ferguson et al. (2018)].

Urinary BPS was correlated with increased gestational age and increased risk of late term birth for girls [Wan et al. (2018)], and with preterm birth [Aung et al. (2019)].

In a EU cohort, no association of bisphenol analogues including BPF with fecundability was reported, but total bisphenols (including 4,4-BPF, BPS, BPB, BPP, BPAF, BPAP, or BPZ) was associated with a longer time to pregnancy in women with inadequate folic acid supplement use [Philips et al. (2018)].

BPF, BPS, BPAF, along with Bisphenol Z (BPZ), Bisphenol E (BPE) and Bisphenol B (BPB) are suspected to be endocrine disrupting chemicals which are oestrogenic [Mesnage et al. (2017)]. In human studies investigating health effects including endocrine effects of BPS and other bisphenols [see review by Pelch et al. (2019)], conflicting results have been reported for an association with obesity, diabetes, fasting blood glucose or insulin resistance for BPA analogs including BPS and BPF. Regarding effects on thyroid, BPS was associated with a suggestive increase in TSH, as well as a decrease in free T4 [Aker et al. (2019)].

In vivo scientific evidences were also released recently on BPS and organs or systems such as the mammary gland, female reproductive system, the male reproductive system and on metabolism and obesity. A developmental toxicity study on BPS according to OECD guideline 414 in pregnant rats did not reveal any reproductive, developmental or teratogenic effects (ECHA Dissemination, 2018). However, different academic papers studied the effects of BPS on male reproductive function and suggest a coherent picture of the alterations in spermatogenesis after BPS exposure in both rat and mice [Shi et al. (2018), Horan et al. (2018), Shi et al. (2017), Ullah et al. (2018), Shi et al. (2019)].

Among evidenced effects reported on female reproductive study in animals, are alteration of the pattern of oocyte maturation/meiosis and/or folliculogenesis, and, in a lesser extent, the timing of puberty. [Shi et al. (2017 and 2019), Nourian et al. (2017), Ijaz et al. (2019), Ahsan et al (2018), Horan et al. (2018), Nevorat et al. (2018)].

BPB, BPE, BPF display anti-androgenic activities in some settings [Rosenmai et al. (2014)]. Moreover a study on BPS and BPAF exposure showed that it can modify the histology of zebra fish

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testes and ovaries and influence homeostasis of testosterone and oestradiol, and parental exposure to environmentally relevant concentration of BPAF results in delayed hatching of the offspring [Shi et al. (2015)]. BPS and BPF induce proliferation and migration of breast cancer cells via the oestrogen receptor dependent pathway in vitro [Kim et al. (2017)].

A new text-mining tool was developed to explore the literature and attempt link bisphenols to adverse outcome pathways (AOP-helpFinder). Using this tool as well as systems biology approaches, it was found that BPS could be linked to pathways leading to obesity [Carvaillo et al. (2019)] and BPF to AOP networks leading to thyroid cancer [Rugard et al.(2020)]. These data and others suggest that the safety of BPA substituents is not clear at this stage.

9.1.2 Exposure characteristics

BPA is used in certain plastics, epoxy resins and thermal papers and is among the highest volume of chemicals produced world-wide. There is evidence that contamination can occur through different routes, including food, water, air and skin (particularly in occupational exposure of cashiers). BPA has a relatively short half-life (hours); it is conjugated and believed to be inactive in that form, but there is concern that it may be locally deconjugated at the tissue level. There is a clear advantage in measuring free and conjugated forms both to address the possibility of external contamination during the assay and to better assess the active form of the substance.

There is solid evidence that a large majority of the human population is exposed to BPA. Many biomonitoring studies are available for bisphenol A (BPA) but the majority of the studies have a single measurement of exposure. These studies are useful in estimating the exposure to BPA in a particular population and follow time trends but not for risk assessment. Studies with multiple biological samples (usually pregnancy cohorts) have shown that BPA has poor Intraclass Correlation Coefficient (ICC) and therefore a single biological measurement can cause exposure misclassification. Further, there is a lack of consensus on how to deal with multiple samples in estimating the correct exposure. In addition, not all countries in Europe have biomonitoring data available on BPA. In DEMOCOPHES¹⁷, seventeen European countries participated, but BPA was added for a group of only 6 countries. BPA is analysed in very few European birth cohorts in Germany, Norway, Spain and France [Casas et al. (2013)].

Bisphenol F (BPF), Bisphenol S (BPS), and Bisphenol AF (BPAF) are among the main substitutes of BPA [Chen et al. (2016); Gao et al. (2020); Yang Y et al. (2019)]. Studies in food revealed that BPS and some other bisphenols can be detected besides BPA in a large number of foodstuffs at low concentrations [Vinas et al. (2010); Liao and Kannan, (2013, 2014)].

As part of the national biomonitoring program, the Esteban cross-sectional study has measured, for the first time in the continental French population, the levels of impregnation with bisphenols A, S and F. The measurement of urinary concentrations of bisphenols was based on a subsample of 500 children and 900 adults, aged 6 to 74, included in the study between April 2014 and March 2016. Bisphenols A, S and F were detected in almost all samples; the geometric mean in BPA was 2.25 and 2.69 µg / g creatinine, respectively, in children and adults; equivalent to 0.44 and 0.53 µg / g creatinine for bisphenol S (BPS), and 0.26 and 0.31 µg / g creatinine for bisphenol F (BPF). Impregnation with bisphenols was higher in children than in adults. The results obtained were close to those observed in North American countries [Santé Publique France (2019)].

¹⁷ Demonstration Of A Study To Coordinate And Perform Human Biomonitoring On A European Scale – DEMOCOPHES (2010)
<http://www.eu-hbm.info/democophes>

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9.1.3 Policy relevance

Regulatory measures have been taken at the EU level while additional measures have been taken in certain countries. In the EU, bisphenol A is regulated under REACH (1907/2006/EC). EU law regulates BPA in plastic materials and articles intended to come into contact with food [Commission Regulation (EU) No 10/2011], and since 2011 BPA has been banned from infant feeding bottles across Europe [Commission Directive 2011/8/EU]. In 2018, the EU further restricts the use of bisphenol A in certain food-contact materials. A specific migration limit (SML) for BPA in varnishes and coating has been introduced and the SML for BPA in the Plastics Regulation has been revised. [Commission Regulation (EU) 2018/213]. Additional measures have been taken in several countries. For example, France banned BPA in all food contact materials [French Law No 2012-1442], other countries like Denmark, Belgium and Sweden, banned it in those materials intended for children under 3.

Since 2017 BPA is on the Candidate List of substances of very high concern for Authorisation (SVHC candidates) as it is classified toxic for reproduction. France has prepared a dossier for the identification of BPA as a human ED-SVHC substance, and Germany for the identification as an environmental ED-SVHC substance. In June 2017, ECHA identified BPA as a substance of very high concern (SVHC) due to alleged endocrine disrupting (ED) effects for human health and the environment [ECHA (2017)]. In October 2019, ECHA prioritised BPA for toughest EU restrictions by proposing its use should be subject to prior authorisation.

There are also controversies between different agencies concerning the most protective Total Daily Intake (TDI). Furthermore, BPA is also present in thermal papers and exposure of cashiers has been assessed and led to a proposal for restriction and substitution. Different committees of ECHA have analysed the benefits and costs of restrictions and sent their conclusion to the European Commission. BPA is restricted in the EU in thermal paper since 2016. The ban has taken effect in January 2020, giving companies time to phase it out and find a safer alternative. BPA is being primarily replaced by BPS in thermal paper, however it is likely not a safer alternative. In Switzerland, BPS is banned from thermal paper (at a concentration equal to or greater than 0.02% by weight) as well, as of June 2020.

Currently in the EU, there is a limit on the amount of BPA that is allowed to leach out of toys for children up to the age of three and in any toys that are intended to be placed in a child's mouth. The migration limit has been decreased to **0.04 mg/l in toys [Commission Directive (EU) 2017/898]**.

BPA regulation is actively debated across the world. BPS and BPF are the major BPA substituents with distinct industrial applications. Much less is known about their putative toxicity and their presence in human matrices, although initial studies have indicated that they may display toxic effects that are similar to BPA [Rochester and Bolden (2015), Auerbach et al. (2016)]. ECHA has started the process for a harmonised classification and labelling on reproductive toxicity for BPS¹⁸. The consultation period was closed on February 2020. In addition, a similar consultation recently finished for BPAF¹⁹. Other bisphenol compounds are also manufactured and little is known about their toxicity and diffusion at this stage.

¹⁸ ECHA 2019; [BPS](#) - CLH report Proposal for Harmonised Classification and Labelling Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2

¹⁹ ECHA 2019; [BPAF](#) - CLH report Proposal for Harmonised Classification and Labelling Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2

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9.1.4 Technical aspects

Although BPA (and to a much lesser extent BPS and BPF) have been assayed in several Human Biomonitoring studies there is a need to harmonise procedures for sample handling, storage and analytical methodologies. However, assays for conjugated and free substances should also be harmonised. The same holds true for other bisphenols.

Furthermore, external contamination during sample collection, handling and analysis is an important criteria during the evaluation of studies to be considered both for assigning reference values (HBM values) and risk assessment. For BPF and BPS, there are few biomonitoring studies available (see below) but there is a lack of literature for other bisphenols [Chen et al. (2016)].

9.1.5 Societal concern

In several countries and probably world-wide, BPA has been considered as the typical endocrine disruptor. In many cases, the societal concern towards EDCs is highly connected to the bisphenol case and to the campaigns to regulate BPA. Therefore there is a lot of expectations in this field. It is important to fill the gaps and to attempt to address the uncertainties, because the bisphenol case appears to be emblematic of the EDC. Whatever we achieve with bisphenols will actually be useful for all EDCs and for the role of public authorities in protecting pregnant women and the next generations.

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9.2 Categorisation of Substances

Table 9-1: Substances included in the substance group, listed according to availability of toxicology and human biomarker data, in category A, B, C, D, E substances (see general introduction)

Category	Abbreviation/ Acronym	Systematic name	CAS No.	Regulation
A	BPA	4,4'-isopropylidenediphenol	80-05-7	REACH Annex V; Annex XVII, Entry 66 PACT list entry 13/04/2017: Hazard assessment. Scope: ED. <u>OSH Legislation</u> : Consumer uses, Article service life, Widespread uses by professional workers, Formulation or re-packing, Uses at industrial sites, Manufacture, Signs at work, CAD, Young workers, Pregnant or breastfeeding workers. <u>Environmental legislation</u> : Classified Seveso Waste Directive Annex III <u>Professional and consumer legislation</u> : Cosmetics (EC) No 1223/2009 Annex II; Toy safety Directive Appendix C
C	BPS	4,4'-sulphonyldiphenol	80-09-1	CoRAP list PACT list entry 01/04/2015: Hazard assessment. Scope: ED. <u>OSH Legislation</u> : Article service life, Formulation or re-packing, Uses at industrial sites, Manufacture.
C	BPF	4,4'-methylenediphenol	620-92-8	REACH Annex III Inventory PACT list entry 01/10/2015: RMOA. Scope: ED. Under development <u>OSH Legislation</u> : CAD
C	BPB	4,4'-(1-methylpropylidene) bisphenol	77-40-7	REACH Annex III Inventory PACT list entry 07/03/2017: RMOA. Scope: ED. Under development <u>OSH Legislation</u> : CAD
C	BPAF	4,4'-[2,2,2-trifluoro-1-(trifluoromethyl)ethylidene] diphenol	1478-61-1	REACH Annex III Inventory PACT list entry 01/10/2015: RMOA. Scope: ED. Under development <u>OSH Legislation</u> : CAD <u>Environmental legislation</u> : Classified Seveso

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Category	Abbreviation/ Acronym	Systematic name	CAS No.	Regulation
C	BPAP	4,4'-(1-Phenylethylidene) bisphenol	1571-75-1	REACH Annex III Inventory <u>OSH Legislation</u> : CAD <u>Environmental legislation</u> : Classified Seveso Waste Directive Annex III
C	BPBP	2,2-bis(2-hydroxy-5-biphenyl)propane	24038-68-4	-
C	BPC	4,4'-isopropylidenedi-o-cresol	79-97-0	REACH Annex III Inventory
C	BPCI2	4,4'-(dichlorovinylidene) diphenol	14868-03-2	REACH Annex III Inventory <u>OSH Legislation</u> : CAD
C	BPE	4,4'-Ethylidenebisphenol	2081-08-5	-
C	BPPH	4,4'-Dihydroxytetraphenylmethane	1844-01-5	-
C	BPM	4,4'-(1,3-phenylene-bis(1-methylethylidene))bis-phenol	13595-25-0	CoRAP list PACT list entry 02/02/2017: Hazard assessment. Not ED. <u>OSH Legislation</u> : Article service life, Uses at industrial sites, Manufacture, Signs at work, CAD, Pregnant or breastfeeding workers. <u>Environmental legislation</u> : Classified Seveso Waste Directive Annex III
C	BPP	4,4'-(1,4-Phenylene diisopropylidene)bisphenol	2167-51-3	REACH Annex III Inventory <u>OSH Legislation</u> : CAD
C	BIS2	Bis(2-hydroxyphenyl) methane	2467-09-9	-
C	DHDPE	p,p'-oxybisphenol	1965-09-9	REACH Pre-Registration process <u>OSH Legislation</u> : CAD, Young workers. Waste Directive Annex III

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Category	Abbreviation/ Acronym	Systematic name	CAS No.	Regulation
C	BPFL	9,9-Bis(4-hydroxyphenyl) fluorene	3236-71-3	REACH Registration Dossier <u>OSH Legislation</u> : CAD Environmental legislation: Classified Seveso Waste Directive Annex III
C	BPZ	4,4'-cyclohexylidene bisphenol	843-55-0	REACH Annex III Inventory <u>OSH Legislation</u> : CAD, Pregnant or breastfeeding workers. <u>Environmental legislation</u> : Classified Seveso
C	BP4,4'	Biphenyl-4,4'-diol	92-88-6	REACH Registration Dossier <u>OSH Legislation</u> : Widespread uses by professional workers, Uses at industrial sites, CAD, Young workers. <u>Environmental legislation</u> : Classified Seveso Waste Directive Annex III <u>Professional and consumer legislation</u> : Plastic contact with food (EU) No 10/2011 Annex I

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9.3 Policy-related questions

There are several critical questions concerning bisphenols that need to be resolved. The first is whether different regulations in different countries lead to different internal exposure values and whether the increasingly frequent use of substituents has led to increased exposure and to the presence of mixtures of bisphenols in humans. The second is identify safety values taking into consideration the accumulating knowledge on Bisphenol toxicity particularly at low doses. A third question is whether substitutes are safer than BPA considering their hazardous properties and current and expected exposure to those compounds.

Specific policy-related questions are:

1. What is the current exposure of the EU population to BPA, BPS and BPF?
2. Do different regulatory controls across the EU concerning in particular BPA lead to different exposures?
3. Are bisphenols (BPA and substitutes) exposure levels of concern for health?
4. Is occupational exposure of cashiers (BPA and substitutes) a health concern?
5. What is the toxicity of BPA substitutes?
6. Are health risks age and gender dependent?
7. Can we find evidence for low-dose effects within mixtures?
8. How can HBM feed into assessment of the Tolerable Daily Intake (TDI) for BPA, as set by the European Food Safety Authority (EFSA)?
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?

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9.4 Research Activities to be undertaken

Table 9-2: Listing of research activities to be carried out to answer the policy questions summed up in 1.3

Policy question	Substance	Available knowledge	Knowledge gaps and activities needed
1. What is the current exposure of the EU population to BPA, BPS and BPF?	BPA	Different types of studies could be considered to address the current exposure of the EU population to BPA: well characterised samples such as Cophes/Democophes, high quality national studies, studies including several samples per individual to account for intra-individual variability, studies with available or planned health outcomes. In the annex to table 2 following EU countries are listed to have HBM data on BPA: De, Be, Fr, At, Cz, Se, El and DEMOCOPHES countries. Age groups for which data are available differ among the countries.	Inventorying available HBM data in EU ->WP7 Use already available biomonitoring data in Europe to: 1. Find out which countries lack HBM data 2. Evaluate the quality of the available data such that design of future biomonitoring studies can be improved accordingly, calculate exposure levels. -> WP8, WP9, WP10 To use already available biomonitoring data in Europe to define the minimum number of samples required per individual to estimate the correct exposure to BPA. Ensure that aggregated data are available in IPCHEM and calculate reference value for BPA base on existing data-> WP10; Q:1,2 To compare single samples vs multiple samples for exposure assessment -> WP9, WP13 To identify exposure pathways for BPA and its toxicokinetic characteristics. -> WP12
2. Do different regulatory controls across the EU concerning in particular BPA lead to different exposures?	BPA (Cat. A)	On 16 June 2017 ECHA ²⁰ classified BPA as an endocrine disruptor and a substance of high concern. Specific countries such as France, Denmark and Sweden have already stricter bans in place. ECHA ²¹ , has recently updated the BPA entry to reflect an additional reason for inclusion due to its endocrine disrupting properties causing adverse effects to the environment.	Find out whether there are HBM data or suitable samples available before and after the ban in Fr, Se, Dk? If not, design an appropriate study to analyse samples time trends-> WP7, WP8 Increase knowledge on time trends, geographical comparisons, exposure determinants of existing HBM data and new HBM data (WP9/WP10; Q: 1, 2) Complete the analysis of collected samples from the alignment study and initiate data exploitation and analysis. HBM data for BPA, BPS and BPF (WP8/WP9/WP10; Q: 1, 2)

²⁰ <https://echa.europa.eu/-/msc-unanimously-agrees-that-bisphenol-a-is-an-endocrine-disruptor>

²¹ <https://echa.europa.eu/-/seven-new-substances-added-to-the-candidate-list-entry-for-bisphenol-a-updated-to-reflect-its-endocrine-disrupting-properties-for-the-environment>

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Policy question	Substance	Available knowledge	Knowledge gaps and activities needed
3. Are bisphenols exposure levels of concern for health?	BPA (Cat. A) BPS, BPF (Cat. C)	KEMI ²² has identified a total of over 200 chemical substances that have a chemical structure similar to Bisphenol A and which may be found on the European market. According to results from data simulations, 37 of these substances may have endocrine disrupting properties like those identified for Bisphenol A. Only available in vitro/in vivo experimental settings in which BPA AOP have already been explored will be used to assess the effects of BPS and BPF.	To determine whether current or expected exposure levels of BPS and BPF are of concern for health in the general population and at the workplace? Derive health based HBM guidance values for the general population and for workers (WP5 / T5.2) and to identify the relationship to the environment and workplace. What is the evidence for low-dose effects? Analyse data from longitudinal cohort studies -> WP13 Do BPS and BPF act on the same AOPs as BPA? -> WP13 Targeted assessment of toxic effects of BPS/BPF as compared to BPA. Targets priority will be given to cancer, reproductive, hormonal, metabolic, immune and neurological effects. The linkage with effect biomarkers could be explored in human samples as well as mixture effects. -> WP13 / T13.1, WP14 / T14.3 and WP15 / T15.3. Urgently harmonise procedures for sample handling, storage and analytical methodologies for BPA, BPS and BPF to minimise external contamination. Encourage European countries to participate in inter-laboratory comparisons. Optimise the analytical method for BPS and BPF. Establish the definitive database with those labs which successfully achieved quality criteria for bisphenols assays (BPA, BPS and BPF) -> WP9 To identify existing analytical methods allowing to monitor in human matrices BPA, BPS, BPF and possibly other BPs, as well as the necessary gaps to be fulfilled in terms of method development and validation. -> WP9, WP16 To identify exposure pathways for BPS, BPF (possibly other BPs) and their toxicokinetic characteristics. -> WP12 To identify effect biomarkers associated to bisphenol exposure and to determine whether those effect markers are common to all bisphenol compounds. -> WP14

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Policy question	Substance	Available knowledge	Knowledge gaps and activities needed
4. Is occupational exposure of cashiers a health concern?	BPA (Cat. A)	BPA is restricted in the EU in thermal paper since 2016 ²³ . The ban takes effect in 2020. A directive ²⁴ revised the OEL (Occupational Exposure Limit values) for BPA of 2mg/m ³ TWA in occupational settings.	To find out whether BPA occupational exposure of cashiers is a health concern. To feed into the Commission decision on whether to ban BPA in till receipts, as recommended by ECHA's Committee for Socio-Economic Analysis (SEAC).-> WP8 Finalise external and internal modelling predictions for bisphenol A, F and S (WP12; Q: 2, 3, 4, 8)
5. What is the toxicity of BPA substitutes?	BPS, BPF (Cat. C)	BPS and BPF are the primary substitutes of BPA. Some countries have started the process to restrict BPS because of its toxicity profile. DG Grow and DG Santé recommend to monitor BPA as well as BPS and BPF, the most prominent substituents. Recent findings in the US ²⁵ have shown that some people's exposure to BPF can meanwhile be higher than to BPA. This evidence should also be explored in Europe particularly in women of child-bearing age.	What is the toxicity of BPA substitutes? To identify effect biomarkers associated to bisphenol exposure and to determine whether those effect markers are common to all bisphenol compounds. -> WP14 Finalise the studies on Adverse Outcome Pathways (AOPs) for BPA, BPF, BPS, identify gaps and apply new approaches linking those chemicals to AOPs such as quantitative AOPs (WP13; Q: 3, 5, 6, 7) Exploit cohorts based on existing data and summarise links between bisphenols and health outcomes. Focus on male fertility endpoints and endocrine disruptive effects. (WP13; Q: 3, 5, 6, 7)
6. Are health risks age and gender dependent?	BPA BPS, BPF (Cat. C) Other Bisphenols (Cat. C)	Most regulation and recommendation tend to focus on pregnant women and infants.	To determine age and gender specific health effects of BPA. -> WP9, WP10, WP13 To derive health based HBM guidance values and perform risk assessments for different age groups and sex. -> WP5 / T 5.2 & 5.3 To measure hormonal levels in new-borns exposed transplacentally and pubertal children due to environmental exposure to BPs. -> WP14

²² <https://www.kemi.se/en/news-from-the-swedish-chemicals-agency/2017/new-report-37-bisphenols-may-be-endocrine-disruptors/>

²³ <http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32016R2235&from=FR>

²⁴ <http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32017L0164&from=FR>

²⁵ <https://silentspring.org/blog/results-our-biomonitoring-study-are>

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Policy question	Substance	Available knowledge	Knowledge gaps and activities needed
7. Can we find evidence for low-dose effects within mixtures?			To determine the effect of combined exposures to substance mixtures within the bisphenol family and with other families and whether this could impact health guidance (in food contamination, cosmetics, other plasticizers, etc.) -> WP15 To identify effect biomarkers associated to bisphenol exposure and to determine whether those effect markers are common to all bisphenol compounds. Assay selected effect markers in the alignment studies and link them to BP levels -> WP14 To develop indicators for combined exposures. -> WP5 / T 5.4
8. How can HBM feed into assessment of the Tolerable Daily Intake (TDI) for BPA, as set by the European Food Safety Authority (EFSA)?	BPA (Cat. A) BPS, BPF Other Bisphenols (Cat. C)	In its 2015 re-evaluation of BPA exposure and toxicity, EFSA (European Food Safety Authority) used a more refined methodology than before supported by new data to review the Tolerable Daily Intake (TDI) for BPA from 50 to 4 µg/kg bw/day.	To derive EU-wide health based guidance values for BPA and other bisphenols (BPS and BPF). Find out how to feed this into an assessment of the TDI for BPA as set by EFSA. -> WP5 / T 5.2, Finalise the establishment of HBM guidance values at European scale (WP5;Q: 8, 9) To relate exposure pathways including food pathway for BPA, BPS, BPF (possibly other BPs) to HBM data. -> WP12 To determine whether different regulatory controls across EU MS lead to different exposures.
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?	BPA (Cat. A)	As long term goal, it will be 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. Policy proposals should be developed to extend the focus to the whole group of bisphenols.	It is not clear whether these questions can be tackled within HBM4EU: Gather data on environmental persistence and the fate of bisphenols to determine exposure risks to humans and ecosystems. Studies that investigate photo-degradation and microbial degradation would provide understanding of environmental transformation products and fate of bisphenols. Correlate environmental monitoring data with HBM4EU data -> WP12 Use of available data (IPCHEM data) for improved exposure assessment and health impacts of bisphenols (WP5; Q: 8, 9)

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Annex to table 9-2: Listing of available knowledge related to policy questions

Substance	Available knowledge related to policy question
BPA	<p>Germany 1995-2009,(20-29 yr.- urine & plasma): [Koch et al. (2012)] & 2003-2006 (GerES IV); (3-14 yr.- urine): [Becker et al. (2009)] (<i>rp</i>);</p> <p>Belgium, Flanders 2007-2012 (14–15 yr.): [Geens et al. (2014)] (<i>rp</i>) & 2011-2012 (DEMOCOPHES), (6-12yr., mothers, pregnant women- urine): [Covaci et al. (2015) and 3XG (2013)] & 2012-2015 (FLEHS 3), (50-65 yr.-urine): [Steunpunt Milieu en Gezondheid (2015)];</p> <p>Norway 2012,(food & estimated dietary exposure in adults): [Sakhi et al. (2014)];</p> <p>Greece 2009-2011, (mother-child pairs: 2yr., pregnant women- urine): [Myridakis et al. (Oct. 2015)] & 2011-2014, (children <18yr., adults- hair): [Tzatzarakis et al. (2015)] & 2012 (2.5-87 yr. X=49yr.-urine) [Asimakopoulos et al. (Feb. 2014)] & 2014, (adult males, anonymous individuals- urine, serum)- analytical method: [Myridakis et al. (Feb 2015), Asimakopoulos and Thomaidis (2015) and Asimakopouloset al. (Jan. (2014))] & 2014 (Developing foetus, neonates, infants, children and adults- plasma, urine) -continuous lifetime model: [Saringianis et al. (2014)];</p> <p>Austria 2008-2011, (mother- children pairs: 6-11yr., 25-50 yr.-urine): [Hohenblum et al. (2012)] & 2010-2012, (6-15 yr., 18-64 yr., 65-79 yr.-urine): [Hartmann et al. (2016)];</p> <p>Sweden 2008-2009 (food, young women-serum): [Gyllenhammar et al. (2012)] & 2010-2011, (18-80 yr.-urine): [Bjeremo et al. (2013)] & 2010-2013, (17-19 yrs.-urine)-time series:[Jönsson et al. (2014)] & 2011-2012 (DEMOCOPHES) (mother-child pairs: 6-11yr.,<45yr.-urine): [Larsson et al. (2014)] 1996-2011, (first-time mothers-blood serum): [Gyllenhammar et al (2012) Tidstrend 1996-2011]</p> <p>Czech republic 2015, (35.8±4.7 yr.-plasma, seminal plasma) analytical method: [Vitku et al. (2015)] & 2000-2006, (canned foodstuffs, migration)-analytical method: [Poustka et al. (2007)] & 1999-2000 (water samples & river sediments): [Stachel et al. (2003)];</p> <p>France 2011, 2013 (Blood, urine, amniotic liquid, tissue extracts) - analytical method: [Lacroix et al. (2011), Viguie et al. (2013) and Gayrard et al. (2013)] & 2013-2016, (Mother-premature infants-human breast milk): [Deceuninck et al. (2015)] & 2003-2006, EDEN cohort (pregnant women-urine): [Philippat et al. (2014)] & 2011 ELFE cohort (pregnant women on delivery-urine) [Dereumeaux et al. (March 2016) and Dereumeaux et al. (Dec. 2016)].</p> <p>France 2014 -2016 Esteban (children & adults, 6-74 yrs.- urine) [Santé publique France, (Septembre 2019)].</p> <p>Denmark 2018 (children 8.5–16.1 yr.- urine) [Carlsson et al. (2018)] & 2014 (young men- urine, blood & semen) [Lassen et al. (2014)] & 2006-2012 (children, adolescents, young men, and pregnant women- urine) [Frederiksen et al. (2014)] & 2013 (rural & urban mother-child pairs; urine) & (children and adolescents; urine) [Frederiksen et al. (2013)]</p> <p>Spain 2019 (adults, serum) subcohort of the Spanish European Prospective Investigation into Cancer and Nutrition (EPIC) [Salamanca-Fernández et al. (2020)]</p>
BPS	<p>Belgium, Flanders 2012-2015 (FLEHS 3), (50-65 yr.-urine): [Steunpunt Milieu en Gezondheid (2015)] method development;</p> <p>Sweden 1996-2011, (first-time mothers-blood serum): [Gyllenhammar et al (2012) Tidstrend 1996-2011];</p> <p>France 2013-2016 (Mother-premature infants-human breast milk): [Deceuninck et al. (2015)];</p> <p>France 2014 -2016 Esteban (children & adults, 6-74 yrs.- urine) [Santé publique France, (Septembre 2019)].</p>

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Substance	Available knowledge related to policy question
BPF	<p>Sweden 1996-2011, (first-time mothers-blood serum): [Gyllenhammar et al (2012) Tidstrend 1996-2011];</p> <p>Czech republic 2000-2006, (canned foodstuffs, migration)-analytical method: [Poustka et al. (2007)] & 1999-2000 (water samples & river sediments): [Stachel et al. (2003)];</p> <p>France 2013-2016 (Mother-premature infants-human breast milk): [Deceuninck et al. (2015)];</p> <p>France 2014 -2016 Esteban (children & adults, 6-74 yrs.- urine) [Santé publique France, (Septembre 2019)].</p>
BPB, BPAF, BPBP, BPC, BPC12, BPE, BPPH, BPM, BPP, BIS2, DHDPE, BPFL, BPZ, BP4,4'	<p>France 2013-2016 (Mother-premature infants-human breast milk): [Deceuninck et al. (2015)];</p>

rp = representative for the (respective) population

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

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10.1 Background Information

10.1.1 Hazardous properties

Cadmium

Cadmium is a potentially toxic metal that ranks 7th on the priority list of hazardous substances of US Agency for Toxic Substances and Disease Registry's (ATSDR). International Agency for Research on Cancer (IARC) has classified cadmium and cadmium compounds as human carcinogens (Group 1). Chronic occupational exposures (~45 years) to Cd in the air at concentrations of 5-10 µgCd/m³ could lead to renal tubular damage in some of exposed workers and exposure to higher levels of 100 µgCd/m³ may result in obstructive lung disease (Nordberg et al., 2015). Experimental studies showed that Cd can induce lung and prostate cancer in laboratory animals and some epidemiological studies have also found increased rates of cancer in the same and some other organs (Nordberg et al., 2015).

Kidneys, as a major location of Cd accumulation, are primary organ of adverse metal effects that occur at general population after lifelong exposure resulting in urine concentrations of 4 µg Cd/g creatinine. The same level of exposure in more sensitive groups (pregnant and postmenopausal women, elderly) can also lead to bone effects such as osteoporosis and increased risk of fractures. The existence of Cd adverse effects at lower environmental exposures (<1 µg Cd/g creatinine) - related to bone diseases, effects on kidney functions, effects on endocrine system, reproduction and development ect. - has been recently seriously questioned (Åkesson et al., 2014; Nordberg et al., 2015; Apostoli and Catalani 2015; Bernard, 2016).

However, Cd co-exposure and effects in mixtures of chemicals has not been addressed sufficiently. Most experimental and human studies are dealing with exposure to a single element while real environmental exposure is generally characterised by many substances in unpredictable combinations or exposure conditions and by essential metal status (Apostoli and Catalani 2015, Nordberg 2015).

Hexavalent Chromium

Chromium can exist in oxidation states ranging from -2 to +6, but is most frequently found in the environment in the trivalent (+3) and hexavalent (+6) oxidation states. The +3 and +6 forms are the most important as the +2, +4, and +5 forms are unstable and are rapidly converted to +3, which in turn is oxidised to +6 (Towill et al. 1978). Hexavalent form - Cr(VI) - is more toxic than trivalent form.

- ▶ Cr(III) for its high oxidising potential - and easily penetrates biological membranes.

Hexavalent chromium was classified by IARC as a human carcinogen (Group 1) associated with increased lung cancer risk among workers in certain industries and also cancer of the nose and nasal sinuses.

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In EU the estimated number of Cr(VI)-exposed workers in 2012 was ~786,000, with the largest numbers exposed to welding (IARC, 2012). In the EU CLP Regulation (EC) No 1272/2008 they are classified as genotoxic (Muta. 1B) and as carcinogen (Carc. 1B or 1A).

Also the dermal exposure to Cr(VI) compounds can cause skin irritation, ulceration, sensitization, and allergic contact dermatitis (NIOSH, 2002). The toxicity of Cr(VI) in humans has been reviewed extensively (ATSDR, 2012; Costa and Klein, 2006; U.S. EPA 1998). After absorption, mainly via inhalation for workers and/or via ingestion for the general population, Cr(VI) readily penetrates cell membranes. The details of Cr(VI) toxic activity assumed that genotoxicity, including a wide variety of effects such as DNA damage, gene mutation, sister chromatid exchange, chromosomal aberrations, cell transformation, and dominant lethal mutations, may be due to the reduced forms of intracellular origin, formed by the reduction of Cr(VI) to Cr(III) (Stearns et al., 1995). The main protection mechanism against Cr(VI) activity in the lungs and the stomach is the extracellular reduction of Cr(VI) to Cr(III) by a NADPH-dependent mechanism involving ascorbate (De Flora et al., 2000). Animal trials show that glutathione plays an important role in Cr(VI) reduction in erythrocytes, also showing certain reduction activity in the lungs (Suzuki and Fukuda, 1990).

10.1.2 Exposure characteristics:

Natural and anthropogenic sources of Cd (European Chemical Agency, 2013; Nordberg et al., 2015):

Cadmium levels in the environment vary widely and are a consequence of both natural (erosion of parent rocks, volcanic eruptions, forest fires; 10-50 %) and anthropogenic sources (used in : plastics as colour pigment and stabilizer, automobile radiators, alkaline batteries, mining activities, fertilizers, sewage sludge, inappropriate waste disposal; 50-90%). During the twentieth century the world consumption of Cd has increased continuously to a global supply of 22,000 metric tons (International Cadmium Association, 2002) and it has remained at this level since 2000. Cadmium is normally transported between the three main environmental compartments:

Air: Levels of Cd in the ambient air are usually low, whereas indoor air levels can be higher due to cigarette smoking (1 - 2 µg of Cd/ cigarette) and poor ventilation. The document of air quality criteria by World Health Organisation (WHO, 2000) indicates levels of Cd in Europe of 1-10 ngCd/m³ for urban areas and 0.1 – 0.5 ngCd/m³ for rural areas. In more remote areas values of 10 – 100 times lower have been reported and around some Cd-emitting industries the levels could approached 200-600 ngCd/m³.

Water: Cadmium concentration of natural surface water and groundwater is usually <1µgCd/L. Drinking water in general does not exceed concentrations of 5 µgCd/ L, but could be contaminated in some occasions due to the Cd impurities of galvanised pipes, water heaters/coolers or by leakage of Cd into groundwater from dumped Cd oxide sludge.

Soil: In nonpolluted areas Cd concentrations are below 1mgCd/kg of soil. Levels in soil can be increased by either waterborne or airborne Cd. Most of agricultural soils contamination occurs by the use of phosphate fertilizers leading in elevated levels of Cd in crops. In Sweden, the levels of fertilizers have been regulated, but a small increase is still occurring, depending on the region and type of farming.

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Natural and anthropogenic sources of Cr(VI) and Cr(VI) compounds

The occurrence of Cr(VI) is rare naturally. Most of Cr(VI) compounds are man-made (products or by-products) and human-caused Cr(VI) contamination is a result of large industrial emissions (mainly from metallurgical, chemical, and refractory brick industries). Major uses of Cr(VI) compounds include metal plating, manufacture of pigments and dyes, corrosion inhibitors, chemical synthesis, refractory production, leather tanning, and wood preservation (Blade et al., 2007). Due to a lack of internal supply and to demand from the steel industry, the EU has been an importer of Cr ores. The main sources for EU imports in 2006 were South Africa (approximately 80%). Within the EU, Finland was the main producer of Cr in 2006, producing over 99% of the total EU Cr production (219,500 tonnes). A report on a critical raw material profile by the European Commission in 2014 revealed that the forecast average annual demand for Cr growth of 3%-4.5% *per year* (EC Report, 2014, Report on Critical raw materials for the EU

<http://ec.europa.eu/DocsRoom/documents/10010/attachments/1/translations>).

Mobilisation of Cr occurs among the following environmental compartments:

Air: In rural areas air Cr concentration above 10 ng/m³ was uncommon whereas in urban (released from anthropogenic point sources) it was 2-4 times higher than regional background concentrations (WHO, 2003). In particular, air Cr concentrations in urban European areas were found to span 4-70 ng/m³, while in industrial European settings were in the range 5-200 ng/m³ (WHO, 2000).

Approximately one-third of the atmospheric releases of total Cr from anthropogenic sources are believed to be in the Cr(VI) form (ATSDR cap. 6). As a result of smoking, Cr concentrations in indoor air (\approx 1000 ng/m³) may be 10-400 times greater than outdoor concentrations (WHO, 2003). In workplace air, on a national level, many countries experienced a level of exposure to Cr(VI) equal to 1 μ g/m³ (France) and 5 μ g/m³ (Sweden, Lithuania and Denmark).

Water: Surface runoff, deposition from air, and release of municipal and industrial waste waters are sources of Cr in surface waters. The Cr(VI) species can persist in aquatic media as water-soluble complexes, but in presence of organic matter (or other reducing agents) it undergoes reduction to Cr(III). Although total Cr may reach levels greater than 50 μ g/L, in general it is detected at concentrations in the order of few tens of μ g/L or lower. In rainwater, Cr concentrations on average fall in the range 0.2-1 μ g/L, some part of which may be accounted for by Cr(VI). Total Cr concentrations in groundwater and water from drinking water sources/supplies may range from < 1 μ g/L up to a few μ g/L. The presence of Cr(VI) in drinking water and/or its precursors is often consequence of anthropogenic contamination by industrial activity, with levels up to 53 μ g/L in the case of Thiva- Tanagra-Malakasa basin (Eastern Sterea Hellas, Greece). Finally, as water treatment facilities use strong oxidants to potabilise water, in drinking water Cr may easily be present in the form of Cr(VI) (WHO, 2003; EFSA 2104).

Soil: Chromium levels in soils can vary up to three orders of magnitude, reflecting the composition of the parent rock from which the soils were formed and/or local anthropogenic sources (WHO, 2000). Estimated total Cr concentrations in agricultural European soils found that total Cr is quite abundant.

From this study, 2.7% of soils were above the threshold value (100 mg/kg) and 1.1% above the guideline value (300 mg/kg) set by the Finnish Ministry of Environment and about 2 million ha of agricultural land - with special emphasis for Piemonte, Lorraine-Alsace, Western-Macedonia and Central Greece - were considered at an ecological and health risk (Toth et al., 2016).

Other sources of exposure to Cr(VI) need to be considered for general population, including the release Cr, with Cr(VI) as the predominant species, from orthopedic implants made from stainless

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steel and cobalt-chromium alloys. Dermal exposure through leather articles and cosmetics, and oral exposure of children through toys have been reported.

Human exposure route and Human Biomonitoring (HBM) data availability for Cd

General population is exposed to Cd primarily through diet and drinking water (5-10 % of ingested Cd is absorbed), and tobacco smoke (10-50 % of inhaled Cd is absorbed). The mean exposure of adults in Europe and North America through food is 10-20 µg Cd/day, which results in average urinary excretion of 0.5-1.0 µg Cd/day and blood concentrations of 0.5-1.0 µgCd/L for non-smokers (twice as high in smokers) (Nordberg et al., 2015).

At the European level the biomarkers are collected in national HBM programs (German Environmental Survey, GerES; The Flemish Environment and Health Study, FLEHS; French Nutrition and Health Survey, ENNS; Czech Republic HBM, CZ-HBM; Slovenian National HBM; etc.) and international projects (Public health impact of long-term, low-level mixed element exposure in susceptible population strata, PHIME and DEMOnstration of a study to COordinate and Perform Human Biomonitoring on a European Scale, COPHES/DEMOCOPHES).

Health-based reference values for cadmium in urine are 1 µg/L (µg/g creatinine; HBM I) and 4 µg/L (µg/g creatinine; HBM II) for adults, and 0.5 µg/L (µg/g creatinine; HBM I) and 2 µg/L (µg/g creatinine; HBM II) for children, as set by the German Human Biomonitoring Commission (Schulz et al., 2011). In blood, reference value is below 1 µg/L for adults (Wilhelm et al., 2004).

Human exposure route and Human Biomonitoring (HBM) data availability for Cr(VI)

Breathing contaminated workplace air is the main source in occupational setting. For the general population exposure to Cr occurs primarily by ingestion of Cr-contaminated soil, food, and water, but also through inhalation of ambient air. Cigarette smoking is another important source of Cr exposure, including the hexavalent state. When talking about total Cr, it is accepted that the contribution of drinking water to the total exposure is substantial only when levels are above 25 µg/L (WHO, 2003). However, the EFSA Panel on Contaminants in the Food Chain noted that the contribution of drinking water to total Cr refers to Cr(VI), whereas in food the trivalent form Cr(III) is the major form. Mean chronic exposure assessment for Cr(VI) across European dietary surveys through the consumption of drinking water ranged from 0.7 ng/kg b.w. per day to 159.1 ng/kg b.w. per day (EFSA, 2014).

Biological monitoring of exposure to Cr(VI) compounds is a practice in occupational settings. In workers, the distribution of inhaled Cr(VI) permits the biological monitoring of Cr in urine, whole blood, plasma, and blood cells. Relevant biological monitoring guidance values for occupational exposure to Cr have been reported on a national basis, but not at EU level. The Spanish authorities set 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 (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, in order to help interpretation of occupational biomonitoring results, DFG did set a BAR (Biologischer Arbeitsstoff-Referenzwert) for the general not occupationally exposed population of working age of 0.6 µg/L urine for Cr(VI) compounds (inhalable fraction) (DFG, 2012). DFG further established the DFG-EKA values (biological exposure equivalents for carcinogenic substances) for Cr(VI) that set the range of total Cr in urine (from 10 µg/L to 40 µg/L) and in erythrocyte fraction of whole-blood (from 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).

No HBM survey have been performed at EU level on Cr(VI) exposure of the general population. Few Human Biomonitoring data come from individuals accidentally or intentionally ingesting Cr(VI) compounds.

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10.1.3 Policy relevance

Cadmium

Since cadmium is listed in Regulation (EC) No 1272/2008 as human carcinogen, (Carc. 1B) and due to its increasing evidence of toxicity, both national and international agencies have sought to regulate its exposure. The WHO (2004) guidelines for drinking water quality has been revised from 5 to 3 µgCd/L and WHO (2000) guidelines for ambient air 5 ngCd/L. The Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2012) has recommended a maximum intake from food of 25 µg/kg bw/month (Nordberg et al., 2015). The main rationale for action/inaction lies in Regulation (EC) No. 1881/2006 of 19 December 2006 that sets maximum levels for certain contaminants in foodstuffs and contains the most recent maximum levels for Cd in foodstuffs. Furthermore, use of Cd is restricted in certain products (Annex XVII of REACH), among them recycled PVC, the existing allowed limit of which is currently in review. There is also ongoing discussion as regards allowable maximum levels in phosphate fertilizers with a link to acceptable maximum levels in food. Overall, the levels in food, recycled PVC and fertilizers continue to be reviewed by the Commission. An updated scientific basis is therefore of great importance.

Hexavalent Chromium

In the case of Cr(VI) compounds an oral minimal risk level (MRL) of 0.005 mg/kg b.w. *per day* was derived for intermediate (15-364 days) exposure based on haematological effects in rats, while reported in a chronic drinking water study (> 1 year) an oral MRL of 0.001 mg/kg b.w. *per day* was derived selecting as critical effect non-neoplastic lesions of the duodenum (ATSDR, 2012). The WHO derived an oral tolerable daily intake (TDI) for non-cancer effects of 0.9 µg CrVI/kg b.w. *per day* taking into account the data relative to outcome observed in female mice after exposure to sodium dichromate dehydrate in drinking-water (WHO/IPCS, 2013). In a recent document, EFSA provided information on benchmark doses, margin of exposure (MOE) and TDI for the European population (EFSA, 2014).

To date no EU regulation regarding maximum levels of total Cr in food has been established.

A maximum value of 50 µg Cr/L for total Cr both in water intended for human consumption and in natural mineral waters are reported by the Council Directive 98/83/EC and the Commission Directive 2003/40/EC, but no level is available specifically for Cr(VI).

In air, the EU proposed an OEL (occupational Exposure Limit) for the hazardous Cr(VI) of 25 µg/m³; the number of future cancer cases can be most substantially decreased through full compliance with the OEL (<https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:52016SC0152&from=EN>). In addition, the Occupational Safety and Health Administration (OSHA) has established a permissible exposure limit (PEL) of 5 µg/m³ and an action level (AL) of 2.5 µg/m³; both for a 8-hour time-weighted average (TWA) exposure to Cr(VI) (OSHA, 2006). The National Institute for Occupational Safety and Health (NIOSH) has recommended a 10-hour TWA exposure limit for all Cr(VI) compounds of 1 µg Cr(VI)/m³ (NIOSH, 2013).

Hexavalent Cr is included in the revised Annex XIV to the EU REACH Regulation; inclusion in this Annex means that in order to continue to use chromium trioxide and other hexavalent chromium compounds after 21 September 2017, an authorisation will be required.

In addition, since 1 May 2015 a restriction on Cr(VI) in leather is in place (EU Regulation 301/2014) and applicable at EU level (limit of 3 ppm). That threshold is expected to be 80 % effective in reducing the occurrence of new Cr(VI)-related allergic dermatitis cases due to Cr(VI) in leather articles.

Moreover, current migration limits for Cr(VI) are laid down in the Toy Safety Directive 2009/48/EC for ensuring the safety of toys. The current migration limits for Cr(VI) from toys are: 0.2 mg/kg toy for

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scraped-off toy materials, 0.02 mg/kg toy material for dry (powder-like or pliable) toy materials and 0.005 mg/kg toy material for liquid or sticky toy materials, respectively.

Regarding cosmetics, because of its allergenic character, the presence of Cr(VI) is prohibited in cosmetics by a German cosmetics regulation and also by the corresponding new EU Cosmetics Directive (EU) No 1223/2009; the only allowable green colorants are those based on the trivalent form of Cr (chromium hydroxide green ($\text{Cr}_2\text{O}(\text{OH})_4$) and chromium oxide green (Cr_2O_3)).

10.1.4 Technical aspects

(Nordberg et al., 2015, Bernard et al 2016)

Biomarkers related to low environmental cadmium exposure that are currently commonly used are levels of:

- ▶ Cd in urine is usually accepted as biomarker of body burden reflecting long term accumulation, but such definition is limited to occupational or really excessive exposures. At low environmental situations urine Cd levels are influenced by several factors including physiological variations related to normal (age, circadian rhythm) and stress conditions (physical stress, smoking) or silent (undercurrent) pathophysiological conditions. All these factors are affecting kidney pathways and co-excretion patterns of renal functional biomarkers and Cd itself. Co-excretion of Cd and proteins adds uncertainty to the relationship between UCd and the body burden of Cd.
- ▶ Cd in blood /plasma (in most laboratories chemical analyses are not sensitive enough to permit the accurate measurement of plasma or serum). At low Cd levels blood represents recent and past exposure; they cannot be properly distinguished.
- ▶ Cd in placenta is used as indicator of Cd exposure during pregnancy
- ▶ Cd in cord blood is indicating Cd transfer from maternal blood to cord blood
- ▶ Cd in feces - at low doses comparable with urine excretion)
- ▶ Cd in kidney, liver or bone tissues is reflecting Cd accumulation.
- ▶ Renal function biomarkers in urine such as: albumin (Alb) and Immunoglobulin G (IgG) indicating glomerular kidney damage, and N-acetyl-beta-D-glucosaminidase (NAG), α 1-microglobulin (A1M), β 2 microglobulin (B2M), retinol-binding protein (RBP), Kidney Injury Molecule-1 (KIM-1), metallothioneins (MTs) indicating tubular kidney damage (Nordberg et al., 2015) – at low levels they rather function as indicators of normal physiological processes, so they are unrepresentative for Cd risk assessment at low levels.

The most common methods for Cd determination in human matrices are inductively coupled plasma mass spectrometry (ICP-MS), atomic absorption spectrophotometry (AAS) and atomic fluorescence spectrophotometry (AFS). For the *in vivo* determination of Cd in tissues, X-ray fluorescence is used. For the determination of renal function biomarkers in urine the standard nephelometric immunochemical method is used, which is less accurate than the measurement of Cd levels in urine or blood. Therefore, determination of renal function biomarkers in relation to Cd exposure and health risk assessment is more reliable at high Cd exposures (> 4 $\mu\text{gCd/ml}$).

Biomarkers related to Cr(VI) exposure are, currently, the followings:

An important consideration in biological testing for Cr(VI) is the reduction of Cr(VI) to Cr(III) throughout the body. Basically, inhalation is the primary route of concern for occupational Cr(VI) exposure. Inhaled Cr(VI) enters the respiratory system, where it may remain, be reduced, or enter the bloodstream. Cr(VI) may be reduced to Cr(III) in the lungs or plasma and excreted as Cr(III) in the urine. Cr(VI) that is not reduced in the plasma may enter erythrocytes and lymphocytes. This

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distribution of absorbed Cr(VI) permits the biological monitoring of Cr in urine, whole blood, plasma, and red blood cells (RBCs) in occupational settings.

- ▶ Cr in urine

Urinary Cr levels are a measure of total Cr exposure as Cr(VI) is reduced within the body to Cr(III). The average urinary excretion half-life of Cr(VI) is about 39 h.

- ▶ Cr measurements in blood and plasma

Plasma or whole blood Cr levels are indicative of total Cr exposure because Cr(VI) may be reduced to Cr(III) in the plasma. Moreover many variables can affect Cr levels in the blood, including diet, varying rates in gastrointestinal absorption, and individual capacity to reduce Cr(VI).

- ▶ Cr measurements in red blood cells (RBCs)

Intracellular Cr levels are indicative of Cr(VI) exposure because Cr(VI) passes through cell membranes, while Cr(III) does not. The Cr concentration inside erythrocytes indicates exposure to Cr(VI) sometime during the approximate 120-day lifespan of the cells. There are two advantages to the monitoring of Cr levels in RBCs versus urine: i), the sampling time may be relatively independent of the time of exposure, and, ii), it permits the determination of Cr(VI), rather than only total chromium, absorption.

Thus, in principle, erythrocyte Cr concentration was recommended for its specificity but limited by its low sensitivity. Plasma Cr concentration was recommended as a sensitive parameter, limited by its lack of specificity.

- ▶ Cr measurements in exhaled breath condensate (EBC)

In the last years also the exhaled breath condensate (EBC) is depicted as a very good biomarker of occupational exposure. This could potential be a non-invasive alternative to measure exposure to Cr(VI) compounds long after exposure.

In any case, the above biomarkers of exposure are not sufficiently validated and great efforts could be made in this sense. In addition, while biomonitoring of occupationally exposed workers has been used to assess high-level inhalation exposures in the workplace, evaluating low-level environmental exposure to Cr(VI) has to be still addressed.

Moreover, the inter- and intrapersonal variability in background levels of Cr is known to be significant and influenced by food and beverage intake, smoking, exercise, habits. Thus, the role of each factor must be carefully understood.

Overview of the biomonitoring methods is available for total Cr in occupational setting. The DFG proposed two regulatory methods: the first for total Cr in urine, the second for total Cr in whole blood as well as in plasma and in erythrocytes. The analytical determination is done using a standard graphite or a pyrolytically coated graphite tube in combination with electrothermal atomic absorption spectroscopy (EAAS) (detection limit were 0.1 µg /L and 0.5 µg /L) (DFG, 1990). Other analytical techniques for total Cr determination in human matrices is inductively coupled plasma mass spectrometry (ICP-MS).

Because Cr(VI) is largely reduced to Cr(III) in the body, speciation of Cr could not be useful in HBM programmes. However, several methods aiming at direct or indirect measurement of Cr(VI) have been published in literature. They are usually based on some kind of separation of Cr(III) and Cr(VI) (i.e., micro liquid chromatography (µLC) system or ion chromatography), followed by ICP-MS quantification (detection limits ranging from 0.1 to 1.0 µg/L).

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However, as far as know, none of these methods have obtained the status of regulatory method yet nor have they undergone a validation.

10.1.5 Societal concern:

The societal concerns regarding cadmium exposure is mostly due to (European Chemical Agency, 2013; Nordberg et al., 2015):

- ▶ no decrease in soil Cd concentrations and human background intakes in Europe during recent years in spite of improved regulations and guidelines; in local regions and farms even a slight increase has been observed, particularly in Sweden.
- ▶ possible occurrence of adverse effects in susceptible populations at present exposure levels due to continuous accumulation of cadmium in the body
- ▶ uncertainties in health risk assessment and therefore in deriving a safe exposure level,
- ▶ 'high societal costs in terms of health care and shortening of life time and a decreased quality of life' (European Chemical Agency, 2013; Nordberg et al., 2015).

The societal concerns regarding Cr(VI) exposure are mostly related to:

- ▶ anthropogenic Cr(VI) occurrence in water, air and soils as a consequence of industrial activities despite the available limits provided by European regulations and guidelines;
- ▶ occurrence of Cr(VI) in many consumers' products such as leather, toys, cosmetics, despite the limits already in place at European level;
- ▶ some populations are at higher risk for exposure to Cr(VI), such as children (e.g., from toys) and occupationally exposed workers in many industries;
- ▶ possible occurrence of adverse effects with respect to cancer, reproductive and developmental toxicity, but also skin sensitisation and allergy, in exposed and general populations;
- ▶ the absence of harmonised HBM reference levels and toxicological derived guidance values for Cr(VI) at European level and the lack of validated analytical tools;
- ▶ uncertainties in health risk assessment considering also the inter- and intrapersonal variability of Cr(VI) levels and the influence by food and beverage intake, smoking, exercise, habits;
- ▶ high prevalence and incidence of Cr(VI) allergy in the general population and risks of carcinogenic effects, maximise the societal costs in terms of quality of life and health care.

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10.2 Categorisation of substances

Table 10-1: Substances included in the substance group, listed according to availability of toxicology and human biomarker data, in category A and B substances

Cat.	Abbrev./ Acronym	Systematic name	CAS No.	Regulation
A	Cd	cadmium	7440-43-9	Regulation (EC) No 1272/2008 as carcinogen Regulation (EC) No. 1881/2006 for food Restricted use of Cd: Regulation (EC) No. 1907/2006; Annex XVII of REACH (entry 23)
C	Cr(VI)	hexavalent chromium	18540-29-9	Revised Annex XIV to the EU REACH Regulation Directive (EU) No 1223/2009 on cosmetics CLP Regulation (EC) No 1272/2008 as genotoxic (Muta. 1B) and as carcinogen (Carc. 1B or 1A) REACH Regulation (EC) No 1907/2006 for inclusion of substances in the Authorisation List (Annex XIV) Regulation (EU) No 301/2014 in leather articles Directive (EC) No 2009/48 on toy safety

10.3 Objectives / Policy-related questions

Objectives:

1. Synthesise an overview of available biomonitoring and exposure data on Cd and Cr(VI) relevant to the European population.
2. Overview of toxicological data on Cd and Cr(VI) available for European population
3. Identify data and analytical gaps.
4. Identify the key groups at risk considering:
 - ▶ life-style, nutritional status and genetic background,
 - ▶ gender, age; postmenopausal women, elderly,
 - ▶ regions with elevated levels in the environment,
 - ▶ occupational settings,
 - ▶ co-exposure to chemical mixtures.
5. To assess EU exposure to Cd due to use of phosphate-based fertilizers.

Based on the information above, develop a plan for population-based cross-European and/or targeted HBM studies (demonstration studies) within 2-5 years HBM4EU program.

Policy questions related to Cd:

1. What is the current (last 10 years) exposure of the European population to Cd?
2. Does the exposure to Cd differ significantly between countries and population groups? What are the main reasons for differences in exposure?
3. Is there a significant time trend of Cd levels in existing population studies?
4. Is there a link between high soil contamination with Cd and human exposure via dietary sources?
5. Which populations are most at risk?
6. Are the overall exposure levels (in different population groups) above any health-relevant assessment levels (HBM guidance values, TDI)?

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7. Has the regulation under REACH had the favourable impact like a reduction of GM/median concentrations?
8. Are environmental quality standards for Cd in water sufficiently restrictive to protect human health from exposure to Cd via the environment and via dietary sources?
9. Using EU food consumption data, are current Cd food maximum levels sufficiently health protective?
10. Can input be given to the decision as to whether the exemption for Cd in quantum dots under the RoHS Directive can be scrapped?

Policy questions related to Cr(VI):

1. What is the level of exposure to Cr(VI) occupationally relevant in the EU population?
2. Does the exposure to Cr(VI) differ significantly between countries and population groups?
3. What are the groups at risk (e.g. by industrial sector)?
4. Are the exposure levels (in different population groups) above any health-relevant assessment levels (HBM guidance values, BLV, OEL)?
5. Have the newly collected HBM data on Cr(VI) an impact in risk assessment procedures (e.g. REACH)?
6. Which are the appropriate HBM matrices, methods and biomarkers for Cr(VI)?
7. Which are possible health effects resulting from exposure to Cr(VI)?
8. Which are possible mechanistic explanation and AOPs for Cr(VI)?
9. Which are the effects of metals mixtures?

10.4 Research activities to be undertaken

Table 10-2: Listing of research activities to be carried out to answer the policy questions summed up in 7.3

Policy question	Subst.	Available knowledge	Knowledge gaps and activities needed
What is the current (last 5 years) exposure of the European population to Cd?	Cd	Data of various HBM studies across EU	Collection and evaluation of existing data
Does the exposure to Cd differ significantly between countries and population groups? What are the main reasons for differences in exposure?	Cd	Data of various HBM studies across EU; geochemical maps, food consumption data, data on fertilizer use	Geospatial comparison of existing HBM data and its evaluation according to available environmental data (Cd in soil, crops, use of artificial fertilizers,...), and food consumption data.
Is there a significant time trend of Cd levels in existing population studies?	Cd	Data of various HBM studies across EU	Data collected in a defined population group(s) over a certain time period covering at least 3 points.
Is there a link between high soil contamination with Cd and human exposure via dietary sources?	Cd	Content of Cd in soil and various food stuffs in contaminated areas; limited data available on human exposure in contaminated areas.	Assess the link using available data; carry out focused survey(s) to obtain high quality concentration data (soil, food, HBM) and detailed food consumption data.

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Policy question	Subst.	Available knowledge	Knowledge gaps and activities needed
Are the overall exposure levels (in different population groups) above any health-relevant assessment levels (HBM guidance values, TDI)?	Cd	Data of various HBM studies across EU	Comparison of HBM data with TDI values and HBM guidance values
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?	Cd	Content of Cd in water and food stuffs.	HBM data to be used for the reconstruction of exposure/intake dose.
Using EU food consumption data, are current Cd food maximum levels sufficiently health protective	Cd	Data on human exposure; content of Cd in relevant food stuffs.	Reconstruct the intake dose based on the HBM data using reverse dosimetry and estimate the acceptable concentration in relevant food stuffs.
Can input be given to the decision as to whether the exemption for Cd in quantum dots under the RoHS Directive can be scrapped?	Cd	Human exposure and effects data are limited.	Exposure using HBM to be tested in well-designed case studies.
What is the level of exposure to Cr(VI) occupationally relevant in the EU population?	Cr(VI)	Few data available on Cr(VI) levels at workplace in Europe	Implement studies on occupational exposure to Cr(VI) in the EU population
Does the exposure to Cr(VI) differ significantly between countries and population groups?	Cr(VI)	Few data available on Cr(VI) levels in population groups and across EU	Implement studies comparing EU countries and population groups (general population and workers)
What are the groups at risk (e.g. by industrial sector)?	Cr(VI)	Limited data available on Cr(VI) in industries	Investigate and identify groups at risk like heavily exposed workers
Are the exposure levels (in different population groups) above any health-relevant assessment levels (HBM guidance values, BLV, OEL)?	Cr(VI)	Absence of biological monitoring guidance values at EU level	Derive HBM guidance values for occupational Cr(VI) exposure
Have the newly collected HBM data on Cr(VI) an impact in risk assessment procedures (e.g. REACH)?	Cr(VI)	Few data available on Cr(VI) levels after authorisations/restrictions in Europe	Implement the risk assessment evaluation related to Cr(VI)
Which are the appropriate HBM matrices, methods and biomarkers for Cr(VI)?	Cr(VI)	Sensitive analytical methods and specific biomarkers for Cr(VI) are limited	Revise and harmonise/develop analytical methods and biomarkers for Cr(VI)
Which are possible health effects resulting from exposure to Cr(VI)?	Cr(VI)	Health effects data on Cr(VI) are limited	Implement the knowledge on effect biomarkers associated to Cr(VI)
Which are possible mechanistic explanation and AOPs for Cr(VI)?	Cr(VI)	Lack of knowledge on AOPs for Cr(VI)	Find known or suspected AOPs associated to Cr(VI)
Which are the effects of metals mixtures?	Cr(VI)	Mixtures data including Cr(VI) are limited	To be tested in case studies

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11 Prioritised substance group: Diisocyanates

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11.1 Background Information

11.1.1 Hazardous properties

Diisocyanates are a group of chemicals containing two isocyanate functional groups ($R-N=C=O$) in otherwise varied structures. Due to the functional groups, all diisocyanates induce similar health effects, and are potent skin and respiratory tract sensitisers. In addition, carcinogenicity is a concern.

The two major diisocyanates in the European market are 4,4'-methylenediphenyl diisocyanate (MDI), m-tolyldiene diisocyanate (TDI), both of which have several isomers. A third diisocyanate with wide-spread use, especially in car paints, is hexamethylene diisocyanate (HDI). Information on the amounts of their manufacture and/or import in the European Economic Area, as well as their current harmonised health hazard classifications under the CLP regulation, is presented in Table 1. In addition to these three compounds, there are several other diisocyanates in the European market that are manufactured and/or imported in smaller yet notable amounts. Five of them, abbreviated as NDI, XDI, TMXDI, TRIDI and TODI (Table 1) are currently under evaluation for harmonised hazard classification under the CLP regulation in the Risk assessment committee (RAC) of the European Chemicals Agency (ECHA). For these five diisocyanates, there is little information available, and their proposed harmonised classifications are mostly based on read-across from MDI, TDI and HDI. At workplaces, diisocyanates, like MDI, can occur also as oligomers or prepolymers in various products. The chain endings of the oligomers and prepolymers contain, however, free isocyanate groups. In addition, these may also contain traces of monomeric diisocyanates.

The respective degradation products and metabolites of MDI and TDI, 4,4-methylene dianiline (MDA; CAS 101-77-9; 10 000 – 100 000 tonnes per year) and 2,4-toluene diamine (2,4-TDA; CAS 95-80-7; no tonnage information available) are also a concern. They both have harmonised classifications for Skin Sens. 1, Muta. 2 and Carc. 1B, and 2,4-TDA in addition as Repr. 2 (suspected of damaging fertility). Moreover, both MDA and TDA have been listed as substances of very high concern (SVHC) under REACH.

Mixture effects appear highly possible for diisocyanates, particularly concerning the sensitising properties. This is based on their shared mode of action that is a consequence of the protein reactivity of the isocyanate groups, although differences in potency among diisocyanates are likely.

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Table 11-1: Selected diisocyanates in the European market (Source: European Chemicals Agency, <http://echa.europa.eu/>)

Abbrev.	Chemical Name	CAS No.	Manufacture and/or import in the European Economic Area (amount, tonnes per year)	Harmonised health hazard Classification under the CLP regulation (Annex VI of Regulation (EC) No 1272/2008)
MDI	4,4'-methylenediphenyl diisocyanate + other isomers	101-68-8	100 000 – 1 000 000	Skin Irrit. 2, Eye Irrit. 2, Skin Sens. 1, Acute Tox. 4 *, STOT SE 3, Resp. Sens. 1, Carc. 2, STOT RE 2 *
TDI	4-methyl-m-phenylene diisocyanate + other isomers	584-84-9	100 000 – 1 000 000	Skin Irrit. 2, Eye Irrit. 2, Skin Sens. 1, Acute Tox. 2 *, STOT SE 3, Resp. Sens. 1, Carc. 2
HDI	hexamethylene diisocyanate	822-06-0	10 000 – 100 000	Skin Irrit. 2, Eye Irrit. 2, Skin Sens. 1, Acute Tox. 3 *, STOT SE 3, Resp. Sens. 1
IPDI	3-isocyanatomethyl-3,5,5-trimethylcyclohexyl isocyanate + other isomers	4098-71-9	10 000 – 100 000	Skin Irrit. 2, Eye Irrit. 2, Skin Sens. 1, Acute Tox. 3 *, STOT SE 3, Resp. Sens. 1
HDMI	4,4'-methylenedicyclohexyl diisocyanate	5124-30-1	10 000 – 100 000	Skin Irrit. 2, Eye Irrit. 2, Skin Sens. 1, Acute Tox. 3 *, STOT SE 3, Resp. Sens. 1
NDI	1,5-naphthylene diisocyanate	3173-72-6	1000 – 10 000	Skin Irrit. 2, Eye Irrit. 2, Acute Tox. 4 *, STOT SE 3, Resp. Sens. 1 + Addition of Skin Sens 1A and modification of Acute Tox 4 * to Acute Tox 2 under evaluation
XDI	1,3-bis(isocyanatomethyl)benzene	3634-83-1	1000 – 10 000	n/a Skin Sens. 1A and Resp. Sens. 1 under evaluation
TMXDI	1,3-bis(1-isocyanato-1-methylethyl)benzene	2778-42-9	100 – 1000	n/a Skin Sens. 1A and Resp. Sens. 1 under evaluation
TRIDI	2,4,6-triisopropyl-m-phenylene diisocyanate	2162-73-4	100 – 1000	n/a Skin Sens. 1 and Resp. Sens. 1 under evaluation
TODI	3,3'-dimethylbiphenyl-4,4'-diyl diisocyanate	91-97-4	10 – 100	n/a Skin Sens. 1A and Resp. Sens. 1 under evaluation

* Indicates that manufacturers or importers must apply at least this minimum classification, but must classify in a more severe hazard category in the event that further information is available which shows that the hazard(s) meet the criteria for classification in the more severe category (See Annex VI, Section 1.2.1 of the CLP Regulation)

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11.1.2 Exposure characteristics

Diisocyanates are widely used in different applications in industry, most notably in the manufacturing of polyurethanes (that are used for various purposes) and as hardeners in industrial paints, glues, varnishes and resins. Total amounts used in the EU are 2.5 million tonnes per year and MDI, TDI and HDI account for more than 95% of this total volume. Since there are no suitable alternatives for the majority of the uses, the use is not expected to decline in near future.

Also consumers may be exposed to diisocyanates from products containing diisocyanates, particularly glues. In addition, consumers may be exposed from use of large amounts of polyurethane foams in do-it-yourself applications, and from on-site-construction activities in public and private buildings. Occupational diisocyanate exposure occurs primarily via inhalation and skin, but also through the gastro-intestinal tract. TDI and HDI are relatively volatile, and their air concentrations can, therefore, be significant at room temperature. Also other diisocyanates, such as MDI, can reach high air concentrations in certain conditions of use, for instance during spray painting. Furthermore, heating of products containing polyurethans can produce diisocyanate monomers (relevant e.g. in welding, soldering, flame cutting and sawing).

For the major diisocyanates, particularly MDI and TDI, there are HBM data available from polyurethane manufacturing, and also some data from the construction sector. Most of the studies are, however, mainly focused on large companies such as polyurethanes industries or paint factories in which the protective personal equipment (PPE) and the safety procedure are often well established. Less data is available from small/medium companies (SMEs) or micro-sized companies (car painting shops, construction painters, etc.) where the exposure of workers can be more relevant due to reduced attention of workers regarding the safety procedures and the correct use of PPE (Geens et al., 2016; Johansson et al., 2015). Limited biomonitoring data are available on exposure to HDI or NDI.

For IPDI, XDI, TMXDI, TRIDI and TODI, there does not appear to be published biomonitoring data from the past ten years. In addition, health based guidance values have not been determined for diisocyanates, as according to the current view, a threshold value for the sensitising effects does not exist.

11.1.3 Policy relevance

Due to the sensitising properties, the three MDI isomers are restricted under the REACH regulation, and shall not be placed on the market as a constituent of mixtures in concentrations \geq 0.1% by weight for supply to the general public, unless the packaging contains protective gloves and is marked properly (Entry 56 in Annex XVII of Regulation (EC) No 1907/2006).

In addition, the use of MDI, TDI and HDI has been recently proposed to be restricted in the EU unless specific conditions for workers training and risk management measures apply (RAC/SEAC, 2017/2018). The aim of the restriction is not, however, to ban the use of diisocyanates but rather to improve the control of diisocyanate use by obligatory training for good working practices and risk management. This is the first time that this type of restriction has been proposed at the EU level and there is an interest to follow-up on the effectiveness of the restriction. If the restriction proposal on diisocyanates is going to come in force, it should have an impact on the exposure to diisocyanates, but the SMEs may still pose a challenge. Therefore, a follow-up on the effectivity especially in SMEs is of high interest.

Also an MDI metabolite, MDA, has been placed in the list of authorised chemicals due to its classification for carcinogenicity (Entry No 02 in Annex XIV of Regulation (EC) No 1907/2006).

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11.1.4 Technical aspects

For Human Biomonitoring, diisocyanate metabolites (diamines) can be measured in urine (for instance, MDA for biomonitoring of MDI, and similarly TDA for TDI). After the hydrolysis of urine, the released amines can be analysed using different methods (LC-ECD, GC-MS, LC-MS/MS). Cocker and Jones (2017) have published a method that allows the simultaneous determination of the metabolites of hexamethylene diisocyanate (HDI), 2,4-toluene diisocyanate and 2,6-toluene diisocyanate (TDI), isophorone diisocyanate (IPDI) and methylene diphenyl diisocyanate (MDI) in human urine. However, challenges have been faced e.g. in the case of workers' co-exposure to both aromatic amines and diisocyanates (Gries et al., 2013, Sabbioni et al., 2000; Cocker, 2011; Jones et al., 2017). Urinary metabolites of MDA reflect both the exposure to MDA and MDI (Tinnerberg et al., 2008; Cocker, 2011; Sabbioni et al., 2010; Jones et al., 2017), which may be an issue in some sectors in which co-exposure may occur (Six and Richter, 2003). Therefore, the relevance of non-isocyanate sources for MDA exposure at the workplace should be clarified before selecting biomonitoring parameters. When using urinary metabolites for the biomonitoring of diisocyanate exposure, also elimination kinetics, which may differ between the different diisocyanates, needs to be considered (Budnik et al., 2011). Wrong timing of the sampling may lead to the underestimation of the exposure. In addition, occasional and lower level exposures, which might still lead to sensitisation, are challenging to be detected.

The timing issue is not anymore so important if another approach for diisocyanate biomonitoring, i.e. adduct analysis is used. Either albumin or haemoglobin adducts has been used for the biomonitoring of diisocyanates. The adducts analysis provides several advantages, among which the most relevant is that their half-life is ranging from about 20 days for albumin adducts up to 120 days for hemoglobin adducts, thus reflecting a chronic constant exposure over a longer period of time than urinary concentrations (Sabbioni et al., 2010). This advantage may, however, apply mainly to workplaces with a continuous exposure pattern. The ability of adducts to detect occasional exposures to sensitising isocyanate concentrations still remains to be established. Disadvantage of the adduct analysis is the need for blood sampling and that they usually need a significant amount of material to work with and involve usually a complex sample preparation. There are specific adducts identified for diisocyanates (e.g. MDI-Lys, AcMDI-Lys) whereas arylamine adducts can be formed both due to the exposure to aromatic amines and isocyanates (e.g. MDA-Val-Hyd, AcMDA-Val-Gly-Gly) (Gries et al., 2013; Sabbioni et al., 2000 & 2010 & 2016). These diamine based DNA adducts are often considered as a first step for genotoxic and carcinogenic effects (Sabbioni et al., 2010; Lindberg et al., 2011).

11.1.5 Societal concern

Diisocyanate-induced skin and respiratory sensitisation have been common occupational conditions, although appear to be declining due to improvements in occupational hygiene. Still, diisocyanate asthma and skin sensitisation are big concerns in occupational health, and are diagnosed in different countries each year. It has been estimated that the incidence of diisocyanate induced asthma is between 16-70 cases per 10000 exposed workers annually, meaning a total of 470-2350 new asthma cases in the EU each year (RAC/SEAC, 2017/2018). They are included in trade union priority list of substances of concern. Although there are occupational exposure limit values for some diisocyanates set in different countries, EU wide values do not exist, and these national values are generally not fully protective from sensitisation.

The effectiveness of the diisocyanate restriction and authorisation requirements under REACH should be monitored, especially concerning occupational exposure. Exposure to diisocyanates at small and medium sized enterprises is a particular concern.

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There is also a need to better understand the occupational exposure routes of isocyanates, e.g. via air, direct skin contact, or via ingestion of aerosols in order to target risk management measures correctly.

In addition, sensitive biomonitoring methods, together with air and skin monitoring methods, are needed for the assessment of the effectiveness of the personal protective equipment.

Furthermore, as diisocyanates can cause occupational asthma and skin sensitisation at very low exposure levels, and the appropriateness and the sensitivity of HBM methods to detect low level exposures may need further development. In addition, it would be important to study exposure to the less known diisocyanates.

11.2 Categorisation of Substances

Table 11-2: Substances included in the substance group, listed according to availability of toxicology and human biomarker data, in category A, B, C, substances (see general introduction)

Category	Abbreviation/ Acronym	Systematic name	CAS No.	Regulation
B	-MDI			Proposed: <u>Annex XVII of REACH</u>
	-TDI			
C	HDI			
	IPDI			
	NDI			
D	XDI			
	TODI			
	HDMI			
	TMXDI			
	TRIDI			

11.3 Policy-related questions

1. What is the current occupational exposure to diisocyanates?
2. What are the best markers to identify hazardous exposures to diisocyanates?
3. What is the likely impact of the forthcoming REACH restriction of diisocyanates?
4. What are the health risks and human health impacts of the current occupational diisocyanate exposures?

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11.4 Research Activities to be undertaken

Table 11-3: Listing of research activities to be carried out to answer the policy questions

Policy question	Substance	Available knowledge	Knowledge gaps and activities needed
1	All diisocyanates	Sporadic information on exposures available, not very recent	<u>Action</u> : Review of the available data on diisocyanates, identification of data gaps (sectors with limited data)
1,2,3	All group a and b diisocyanates	Sporadic information on exposures available, not very recent. Different approaches for biomonitoring available.	<u>Gaps</u> : Exposure in SMEs using diisocyanates for different purposes? Sensitivity of the HBM methods at low exposure levels? <u>Action</u> : Occupational survey focused on sectors with limited data available. This provides a base-line to study the effects of the restriction and should include also testing of different biomarkers for their sensitivity at low exposure levels.
4	MDI, TDI, (HDI)	Sensitisation capacity of diisocyanates known. Also some diisocyanates possibly carcinogenic.	<u>Gaps</u> : What are the risks at current exposure levels. Is there a cancer risk due to formation of respective diamines? <u>Action</u> : Risk assessment based on biomonitoring data taking into account both asthma and cancer risks. This may need also PBPK modelling data.

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12 Prioritised substance group: Emerging Chemicals

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12.1 Background Information

Emerging Chemicals should be understood as chemicals of emerging concern (CECs), which can reach human tissues via direct usage of consumer products or uptake via the environment and food. Most of them are manufactured or manmade and their toxicity or persistence are likely to significantly alter the metabolism of a living being (Sauvé and Desrosiers, 2014). Those substances are not yet included in existing HBM programs, partially due to the absence of analytical method available to determine the considered chemical or its metabolites in human specimen. In any case there is a lack of knowledge about the burden of the general population with these so-called emerging substances.

Chemicals can be considered as emerging substances when: (i) they are really '**new substances**' (e.g. recently developed substitutes for substances currently under regulation or which have been banned) or (ii) substances possibly already present for a while in the environment-food-human continuum, but '**causing a new concern**'. Such new concerns can arise due to sensitivity improvements of analytical methods, allowing the detection at low concentrations of formerly not detected substances in the environment or human. In addition, new application fields developed by the chemical industry for a known chemical can open up a new route of exposure. Alongside, recent toxicological facts including increasing presence in the environment and effects on environmental species can be an alert and can also change the perspective for human risk assessment on a given chemical. At a regulatory and policy level, the main challenge associated to CECs is to develop early warning capability to rapidly handle these chemicals through biomonitoring program and further risk assessment process. At a scientific level, the main challenge associated to the detection of CECs and relevant chemical mixtures is to develop new methodological strategies to rapidly document the reality of exposure and the related health impact for these chemicals, then to detect and prioritise these chemicals on the basis of relevant and well integrated exposure and toxicological data.

In interaction with the prioritisation process established within WP4, a complementary list of emerging chemicals candidates is being generated by WP16. This inventory is based on existing lists of emerging chemicals elaborated at international level (including ECHA, REACH, NORMAN, US EPA...) but also on bottom-up suggestions originated from WP16 partners daily involved in the characterisation of the Human chemical exposome in various contexts. Importantly, this inventory will also include metabolites of the concerned CECs mainly known at a first stage as parent compounds, with the support to biocomputing modelling. This inventory will be shared and crossed with the WP4 related activity, and further prioritisation will occur by considering available exposure, toxicological, and metabolism data as well as analytical considerations.

Besides this *a priori* inventory based approach, the development and application of suspect and non-targeted approaches will be operated within WP16 in the scope of revealing, then identifying, new (i.e. not yet known) markers of exposure related to chemicals of concern for HBM (parent compound or metabolite).

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Suspect screening approaches

"Suspects" are known compounds in terms of chemical name and structure which are expected ("suspected") to be present in a sample. The typical approach applied in this case is large-scale suspect screening aiming to generate semi-quantitative data and contribute to better prioritisation for further targeted developments. The same approaches are also helpful to elucidate the composition of complex mixtures by simultaneously generating exposure data for a wide range of markers from each individual sample. In most cases, analytical standards are not readily available and therefore, relevant analytical methods are not validated and compound identities not definitive. To some extent, suspect screening can be considered an extension of multi-class/multi-residue analysis, whereby some markers may be unambiguously identified and possibly quantified as per a targeted method, while others are mostly qualitatively measured. This qualitative annotation step refers to the assignment of a given compound identity to a signal detected by suspect or non-targeted approaches and relies on the elaboration and implementation of reference libraries to match the generated experimental data with structural descriptors (e.g., m/z, experimental or predicted Rt, MS/MS spectra) indexed from a list of a priori defined chemical compounds. Different levels of confidence for the annotation depending on the availability of chemical descriptors need to be provided.

Non-targeted screening approaches

Non-targeted screening aims to detect "unknown unknowns" compounds without any a priori criteria, to identify potential new markers of exposure and toxicological concern. Generally, sample preparation and data acquisition are similar for suspect and non-targeted screening whereas data analysis/mining are different. Although highly challenging, this approach represents the most promising strategy to advance our knowledge of the human chemical exposome. In addition, it will enable better anticipation of future health threats and related risk assessment and regulatory dispositions. The development and implementation of NTS requires advanced capabilities and good integration of new front-of-science data management aspects (advanced data acquisition and processing facilities, bioinformatics and modelling tools). A solid basic knowledge of chemistry (MS, NMR, chemical synthesis) and biochemistry is essential to allow the unambiguous structural elucidation and relevant interpretation and contextualisation of compounds besides the revealed signals. NTS is then coming with new paradigm modifying the conventional hypothesis-driven research approach to a data generating hypothesis-driven approach, as a really open way to characterise biological samples.

Globally, work on emerging chemicals within the HBM4EU project aims at providing anticipation and early warning, and generating exploratory human data for guiding next orientations of HBM in terms of relevant targets. Concretely the outputs of this dedicated chemical group and associated WP16 are expected to contribute to further in terms of quantitative method development and inclusion of some exposure markers in future HBM programs. This is also referring to a reactivity process and ambition to minimise the delay before warning and real measurement at HBM scale. It is globally based on a principle of reality-driven approach, and a bottom-up characterisation of current human exposome as observed to help prioritisation of further investments and methodological effort targeted toward certain biomarkers of exposure rather than others.

Now all this proposed work in relation with emerging substances still remains a front-of-science associated to a significant level of necessary innovation and methodological research besides these clearly finalised objectives.

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12.2 Categorisation of Substances

Emerging chemicals may fall in two categories.

The first one is related to a priori already identified substances. The second one is related to not yet known/identified substances. For the first category, the prioritisation process and related criteria established within WP4 will be used as a basis for dispatching the different compound candidates between Cat. C and Cat. D. In particular, main criteria considered for this categorisation will rely on (i) the investment needed in term of method development and (ii) the knowledge gap in term of exposure data. Indeed, the total number of substances finally classified into Cat. C after application of the systematic process developed within WP4 is expected to be very high. One part of these substances will be handled in WP9 with regard to the development and/or adaptation of appropriate quantitative methods. But realistically this will not be the case for the whole set of compound candidates. For some of these substances (constituting the Cat. D group), the development and application of a semi-quantitative suspect screening approach is then proposed in WP16, with the objective to generate a first level of data enabling to document the reality of human exposure and better justify further investment in a full quantitative and validated method development.

For the second category (constituting the Cat. E group), non-targeted screening approaches coupled to identification of unknowns capabilities and competences will be developed and applied in order to reveal, and further identify, new (i.e. not yet known) markers of exposure related to chemicals of concern for HBM (parent compound or metabolite). From a methodological point of view, this main component of the WP16 work plan will be based on the last generation of mass spectrometric technologies, that offer a unique and never achieved perspective for such global and untargeted sample characterisation. High resolution mass spectrometry, already in place in several labs in EU, will be the main support of these investigations, coupled to hyphenated competences in terms of data processing and analysis for extracting the relevant information from the generated global chemical profiles.

Table 12-1: Substances included in the substance group, listed according to availability of toxicology and human biomarker data

Cat.	Abbrev./ Acronym	Systematic name	Regulation
A	-	-	-
B	-	-	-
C	-	-	-
D	<i>a priori</i> already identified compounds but not yet measured in humans to be measured by suspect target screening	<u>To be defined as a result of the first year prioritisation process</u>	-
E	substances measured by non-target screening and (1) described in chemical databases or (2) not yet described (unknowns)	-	-

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12.3 Objectives / Policy-related questions

1. Providing early warning of presence of unknown and emerging concern chemicals in EU population
2. Inform REACH process to identify substances of very high concern
3. Inform development of strategy for a non-toxic environment (7th Environment Action Programme)

12.4 Research activities to be undertaken

Table 12-2: Listing of research activities to be carried out to answer the policy questions

Substance	Policy question	Available knowledge related to policy question	Knowledge gaps / Activities needed to answer policy question
D	Early warning of presence in EU population	Different inventories of emerging chemicals exist internationally in the field of environment, food safety, registration of chemicals for the REACH process, occupational exposures	<p>Inventarise existing lists or databases related to emerging chemicals at international level to get a good overview (WP16 Y1)</p> <p>Check whether it is analytically feasible to monitor substances on these lists in human samples.</p> <ul style="list-style-type: none"> ➤ Develop prioritisation tool for analysis of these chemicals based on kinetics and toxicological properties, production volume and policy/societal concerns (WP4). ➤ Improve suspect and non-targeted screening methods to allow detection of emerging chemicals (WP16) including effect directed screening assays (WP14), improve and apply these methods for different human matrices (urine, blood, placenta, maternal milk, adipose tissue, meconium...) including sample preparation, information extraction, data processing and provide guidelines for method validation performance assessment and QA/QC consolidation. ➤ Select biobanked samples for screening. ➤ Screen human matrices for the presence of emerging chemicals. Collate existing data on mammalian metabolism/distribution/excretion of the selected Cat. D emerging chemicals. If not available: predict potential metabolites using computer models/software and existing data as input for the screening above (WP12).

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Substance	Policy question	Available knowledge related to policy question	Knowledge gaps / Activities needed to answer policy question
	Inform REACH process to identify substances of potential concern	REACH uses IT screening tools to get information of potential concern	Provide information on biological half-life in human matrices and if possible also linkage to effect and health outcomes (WP12).
	Development of strategy for a non-toxic environment -> first step		<ul style="list-style-type: none"> ➤ Develop an indicator to monitor in humans the bioaccumulation of the above identified chemicals of potential concern. ➤ Develop an indicator to monitor in humans the decrease of total chemical load of environmental chemicals (WP5).

WP16

E			<ul style="list-style-type: none"> ➤ Improve non-targeted screening methods to detect not yet identified emerging chemicals in human matrices including sample preparation, information extraction, data processing and provide guidelines for method performance assessment and QA/QC consolidation.. ➤ Select biobanked samples for first screening steps. ➤ Screen human matrices (urine, blood, placenta, maternal milk, adipose tissue, meconium...) for the presence of unknowns. ➤ Generate databases for identification of the unknowns in human samples, based on mass spectral information
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12.5 References

1. Sauvé, S and Desrosiers, M (2014): A review of what is an emerging contaminant. Chemistry Central Journal 8:15.
2. Norman network: <http://www.norman-network.net/?q=node/81>

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13 Prioritised substance group: Flame retardants

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13.1 Background information

Flame retardant (FR) is the term given to any compound or mixture added to a consumer product or building material to reduce the flammability and thus improve product safety. Flame retardants can be either chemically-bound to the material of the consumer product, or chemical additives (not bound to the product material). A range of both inorganic and organic FRs are in use; however of concern with respect to HBM4EU are in particular the **synthetic organic flame retardants**. There are three primary types of synthetic organic FRs categorised based on their elemental composition, these being bromine (Br), chlorine (Cl) and phosphate (P).

Since the 1970s, the primary FR compounds used were the polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane²⁶ (HBCDD). However, due to concerns regarding their persistence, toxicity and bioaccumulative potential, these compounds have been added to the Stockholm Convention on Persistent Organic Pollutants (www.pops.int), including the most recent addition of deca-BDE (also called BDE-209, referring to the PBDE with 10 bromines) in 2017. Yet, although these compounds are regulated under the Stockholm Convention and through other regulatory mechanisms, the need for FRs has not decreased and this has led to a broadening of the market for FR compounds, with a wide range of replacement compounds used globally. These replacement compounds are typically brominated, chlorinated and organophosphate compounds, some of which are mentioned below. In the following document, OPE (organophosphate esters), refers to the organophosphate-based FRs, while NBFR (novel brominated flame retardant) refers to the brominated replacements for PBDEs and HBCDD.

13.1.1 Hazardous Properties

PBDEs and HBCDDs have been identified to have a range of adverse health effects, including potential neurotoxic, endocrine, and carcinogenic effects.^{inter alia, 1-3} The toxicity of tetrabromobisphenol A (TBBPA) is also well-studied and it has been identified to have a range of potential hazardous properties.⁴⁻⁷ Early evidence suggests that a number of the replacement FRs may have similar health concerns,⁸⁻¹⁰ and moreover, insufficient evidence exists to evaluate toxicity for many of these new FRs. The toxicity and human exposure of selected FRs has been investigated in individual studies, and aquatic toxicity has received significant attention, but there remain large gaps in toxicity studies of directly applicability to human populations.

Bis(2-ethylhexyl)tetrabromophthalate (BEH-TEBP) and 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (EH-TBB) have been identified as potentially bioaccumulative.¹¹ Decabromodiphenyl ethane (DBDPE) is structurally similar to BDE-209 and hypothesised to have similar toxicity. Triphenyl phosphate (TPHP) is identified by ECHA as very toxic to aquatic life, has been found to affect oestrogen receptor binding activities in zebrafish,¹² and may be associated with altered hormone levels and decreased semen quality in men.¹³ Tris-2-chloroethyl phosphate (TCEP) was also found

²⁶ Actually, six isomers of HBCDD exist. Therefore, sometimes the plural HBCDDs is used as synonymous for HBCDD.

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to affect oestrogen receptor binding activities in zebrafish,¹² may affect neurodevelopment, with multiple mechanisms of toxicity,⁸ and is a possible reproductive toxin.¹⁴ TCIPP may also affect neurodevelopment⁸ and is potentially carcinogenic.¹⁴ Tris(1,3-dichloropropyl)phosphate (TDCIPP) may be associated with altered hormone levels and decreased semen quality in men,¹³ may affect neurodevelopment, with multiple mechanisms of toxicity,⁸ and also may be carcinogenic.¹⁴

The OPEs in particular are seeing significant recent use as FRs, and the levels in consumer products, and in the environment are typically orders of magnitude higher than the brominated and chlorinated FRs.^{15,16} A number of OPEs have evidence of toxic effects in mammals, but generally toxicity data is insufficient, and is a crucial knowledge gap considering the high environmental levels of these compounds. Short-term and long-term toxicological data are needed, including additive or synergistic effects of FR mixtures. Many flame retardants exist in mixtures, e.g., the technical mixtures of the PBDEs, and Firemaster 550, which contains triphenyl phosphate (TPHP), isopropylated triphenyl phosphate isomers (ip-TPP), 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (EH-TBB) and bis(2-ethylhexyl)-2,3,4,5-tetrabromophthalate (BEH-TEBP). In terms of toxicity, the PBDEs have received attention as mixtures and as individual compounds,¹⁷ and there is evidence of Firemaster 550 as an endocrine disrupting compound and obesogen.⁹ However, there is generally little attention given to the toxic effects of the typical mixtures of FRs occurring indoors and to which humans are exposed. Thus, the issue of mixture toxicity is highly relevant to FRs, and remains a large data gap within the toxicological knowledge on FRs.

Further knowledge gaps exist in the area of carcinogenicity, especially for hormonal cancers; there is limited information on long-term and chronic health effects; reproductive health and endocrine disrupting effects also require further investigation. Finally, epidemiological studies that include mixtures of FRs are critical to assess links between exposure and health outcomes.

13.1.2 Exposure Characteristics

FRs are widely used in consumer products and building materials, in particular in electronics, textiles and furnishings, automobiles and other vehicles, building insulation, flooring, appliances and ducting, and studies have identified a range of FRs in all of these product groups.¹⁸⁻²³ The amounts of and types of FRs vary widely even within product groupings, and can be found at up to percentage levels in consumer products, but typically are in the µg/g range.

There is extremely limited information on EU and/or global production of FRs.

The provision of this information is challenging for the following reasons: (1) FR producers maintain proprietary control of the chemical composition of some commercial FR mixtures, and information may not be publicly available; (2) regulations and/or information on commercial production of FRs provided for the EU region may not reflect the use in the EU or the potential for human exposure, since many FRs enter the EU already incorporated into consumer products manufactured in other regions, and chemicals already incorporated into consumer products may not be included in some chemical inventories; and (3) the FR market is rapidly changing in response to regulations and shifts in product requirements, and usage information becomes quickly out of date. Further complexity of information of FRs in consumer products arises from variability in FR mass in the same products due to manufacturing variability or use and complex products such as cars contain a range of FRs with components from global sources.

Human exposure to FRs can occur through a variety exposure pathways, via inhalation, ingestion (either through food or ingestion of indoor dusts, as FRs migrate from products and materials into the indoor and outdoor environment) and dermal exposure, including through direct contact with

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flame-retarded consumer products.²⁴ In addition to use as FRs, a number of these compounds (particularly the phosphorus-based FRs) also act as plasticizers,¹⁴ and thus are also added to synthetic materials for this purpose. The exposure pathways differ based on the compound properties and FR use. For example, while adult exposure to some FRs is primarily through diet, for babies and toddlers, due to the hand-to-mouth behaviour and mouthing of toys, the primary exposure pathway is through ingestion of house dust.²⁵

In general, human exposure to PBDEs is lower in Europe than in North America,²⁶ while evidence from indoor dust and chemical usage suggests higher human exposure to HBCDDs in Europe than in North America based on identified correlations between dust and serum concentrations.^{27,28} The strong interpretations of exposure trends from PBDEs suggest that sufficient biomarker data for other FRs, once obtained, will enable similar improvements in understanding of FR exposure and effects in the European population. Some evidence of regional differences in exposure pathways within Europe for the NBRFs and OPEs,²⁹ however there is no systematic overview of regional differences.

13.1.3 Policy Relevance

A small number of FRs are restricted both within the EU as well as at the international level. PBDEs and HBCDD are restricted under the Stockholm Convention on Persistent Organic Pollutants, and now have limited use. Many replacement/alternative FRs are registered under REACH, however there are currently no regulations for a number of FR compounds. Given the existing regulations on flame retardants both at the international (e.g., Stockholm Convention) and European level (e.g., REACH), HBM4EU can contribute by providing information on the effect of legislative restrictions and bans on concentrations in the European human population, particularly with respect to establishing baseline exposure concentrations for current-use flame retardants. Evaluating and comparing temporal trends for banned/restricted vs. current-use FRs will also allow us to determine if current regulations are effective across the EU, and if the emerging FRs are showing signs of accumulation in the environment or within the European population. For the majority of FRs there are no established safety limits, health-based reference values or guidance values, and limited knowledge of usage volumes due to manufacturer confidentiality.

Of the list of 62 FRs in HBM4EU, 1 is registered under REACH under the 10000-100000 t/y tonnage band, 7 FRs at 1000-10000 t/y and 9 at 100-1000 t/y; 3 FRs are not registered under REACH but listed under CoRAP based on (among others) high aggregated tonnage and wide dispersive use. 28 of the 62 FRs are not registered under REACH.

Of concern is the relative lack of information regarding the use, exposure pathways and toxicity of many of these compounds. The European Food Safety Authority (EFSA) identified 17 brominated FRs which are currently in use and with detectable levels in environmental and/or human matrices, and a further ten brominated FRs that have concentrations >0.1% in consumer products and materials, but lack any information on human and environmental levels or even occurrence at all.³⁰ In conjunction with a lack of exposure data, there also is a lack of physicochemical and toxicological information for many of these compounds, and what information is available for some compounds is based on the chemical properties (e.g., quantitative structure–activity relationship models), and estimates rather than direct evidence. This makes it difficult for regulatory bodies and legislative agencies to make informed decisions. Furthermore, the broad suite of known FRs covers a wide range of structures and properties, meaning that in most cases each individual FR must be independently studied to understand emission, exposure and toxicity. Conclusively, it can be said that large data gaps exist for a wide number of FRs.

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HBM4EU provides a platform to identify geographic patterns and time trends of exposure from existing data sets and to identify and rectify where major gaps exist through additional targeted investigation. This will allow regulatory agencies to identify any FRs that may be of concern and to make informed decisions.

13.1.4 Technical Aspects

Highly lipophilic FRs, particularly those with higher persistence, such as the PBDEs, can be detected in parent compound form in human matrices, most commonly in human serum^{31–33} and breast milk.^{34,35} In contrast, some NBFRs and many OPEs are metabolised in the body, and more commonly used biomarkers of exposure are metabolites detected in urine.^{36,37} However, many of the metabolites are uncertain, and metabolic pathways are only characterised for a limited number of FRs.^{38–43} Biomarkers for many FRs of emerging concern are unknown. Target matrices for biomonitoring for the emerging FRs can be inferred from physicochemical properties of the molecules, considering their structural similarity to better quantified compounds, and/or relying on chemical modelling techniques, but there is a lack of practical measurement data for many compounds. Many biomonitoring studies report high detection frequencies of FR biomarkers in human matrices, but there is little systematic assessment of temporal or spatial trends. PBDEs are one of the few compounds where generalisation of trends and distributions has been made from biomarkers.³³ Quantification of a rapidly increasing temporal trend of PBDEs in maternal milk in Sweden^{44,45} lead to initial concerns regarding human exposure to PBDEs and first regulatory actions.

Analytical methods for PBDEs and HBCDD in serum and milk are relatively well-established, and have been applied around the world.^{33,46–52} Analysis for PBDEs is typically via GC-MS, and instrumental parameters vary in individual methods. Analysis of HBCDD can be via GC-MS or LC-MS, however the GC-MS method has limited accuracy⁵³ and does not allow quantification of individual isomers. LC-MS is strongly recommended for HBCDD. The widespread use of C13-labelled internal standards for both PBDEs and HBCDD allows reliable quantification of these compounds.

Within the replacement NBFRs and OPEs, analytical methods are less established, and recent interlaboratory comparisons have identified large inconsistencies in laboratory performance.^{53,54} As the group of flame retardants is defined by its use, not by its chemical identity, it includes many structurally different chemicals. Thus, analytical methods will differ for certain sub-groups of flame retardants. While the phosphorous flame retardants are a relatively homogenous group, the NBFRs vary greatly. Consequently, methods will have to be optimised for each individual compound. The availability of standards often limits method developments. However, new standards become available each year, and specific interests can be communicated to the producers of analytical standards. Certified reference materials are usually not available, or are not applicable. Older reference materials (e.g., <2000) are not often useful as they do not contain the current complex mixture of FRs that are the replacements for the PBDEs and HBCDD.

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13.2 Categorisation of Substances

Category A are substances for which HBM data are sufficient to provide an overall picture of exposure levels across Europe, and interpretation of biomonitoring results in terms of health risks is possible. These substances have identified toxicity to humans and/or environmental systems, and have been regulated/restricted in view of this. Category B substances have some existing HBM data, but it is insufficient to provide a clear picture of human exposure across Europe. Category C substances have scarce HBM data for the European population and require greater knowledge on toxicological characteristics; some biomonitoring data from outside Europe exists. Category D substances have no HBM data from Europe, but some limited HBM data from outside Europe, which can inform on appropriate methods and target matrices. Category E substances have no HBM data. Of the 62 FRs, 9 are in Category A, 12 in Cat. B, 14 in Cat. C, 12 in Cat. D, and 15 in Cat. E.

A detailed breakdown of the separate categorisation based on the availability of toxicological information and HBM data which was combined to determine the overall categorisation listed in Table 1 is available upon request, along with references to support the categorisation.

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Table 13-1: Substances included in the substance group, listed according to availability of toxicology and human biomarker data, in category A, B, C, D, E substances

Cat.	Abbrev./ Acronym	Systematic name	CAS No.	Regulation
A	BDE-28 ²⁷	2,4,4'-Tribromodiphenyl ether	41318-75-6	Restricted under REACH and listed on Stockholm Convention
A	BDE-47	2,2'4,4'-Tetrabromodiphenyl ether	5436-43-1	Restricted under REACH and listed on Stockholm Convention
A	BDE-99	2,2',4,4',5-Pentabromodiphenyl ether	60348-60-9	Restricted under REACH and listed on Stockholm Convention
A	BDE-100	2,2',4,4',6-Pentabromodiphenyl ether	189084-64-8	Restricted under REACH and listed on Stockholm Convention
A	BDE-153	2,2',4,4',5,5'-Hexabromodiphenyl ether	68631-49-2	Restricted under REACH and listed on Stockholm Convention
A	BDE-154	2,2',4,4',5,6'-Hexabromodiphenyl ether	207122-15-4	Restricted under REACH and listed on Stockholm Convention
A	BDE-183	2,2',3,4,4',5',6'-Heptabromodiphenyl ether	207122-16-5	Restricted under REACH and listed on Stockholm Convention
A	BDE-209	2,2',3,3',4,4',5,5',6,6'-decabromodiphenyl ether	1163-19-5	Restricted under REACH and listed on Stockholm Convention
A	HBCDD	Hexabromocyclododecane	3194-55-6, 25637-99-4, 1093632-34-8	On REACH Authorisation List and listed on the Stockholm Convention
B	TPHP	Triphenyl phosphate	115-86-6	Registered under REACH under the 1000-10000 T/y tonnage band and under CoRAP (suspected ED, consumer use High (aggregated) tonnage, Wide dispersive use)
B	TMPP	Tricresyl phosphate	1330-78-5	Registered under REACH, entered onto CoRAP for evaluation based on High (aggregated) tonnage, Suspected PBT/vPvB, Wide dispersive use.
B	TCEP	Tris-2-chloroethyl phosphate	115-96-8	SVHC (Toxic for reproduction (Article 57c)) all uses require an Authorisation under Annex XIV of REACH from 21/08/2015. Being considered for a restriction under Article 69(2)
B	TCIPP	Tris(1-chloro-2-propyl) phosphate	13674-84-5	Registered under REACH

²⁷ Individual PBDE congeners are included rather than homologue groups (as in previous scoping document) in line with existing analytical methods and HBM data.

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Cat.	Abbrev./ Acronym	Systematic name	CAS No.	Regulation
B	TDCIPP	Tris(1,3-dichloropropyl)phosphate	13674-87-8	Registered under REACH, Entered onto CoRAP for evaluation in 2019 as potential endocrine disruptor
B	TNBP	Tri-n-butyl phosphate	126-73-8	Registered under REACH, Entered onto CORAP for evaluation in 2012 based on CMR, High (aggregated) tonnage, Wide dispersive use
B	TBBPA	Tetrabromobisphenol A	79-94-7	Registered under REACH under the 1000-10000 T/y tonnage band and under CoRAP (suspected PBT/vPvB, endocrine disruptor, consumer use, exposure of environment, etc.)
B	TBOEP	Tri(2-butoxyethyl) phosphate	78-51-3	Registered under REACH under 1000-10000 T/y tonnage band
B	BEH-TEBP	Bis(2-ethylhexyl)tetrabromophthalate	26040-51-7	Registered under REACH under the 100-1000 T/y tonnage band and under CoRAP (suspected PBT/vPvB and ED, Other hazard based concern, Exposure of environment, Wide dispersive use)
B	EH-TBB	2-ethylhexyl-2,3,4,5-tetrabromobenzoate	183658-27-7	None
B	BTBPE	1,2-bis(2,4,6-tribromophenoxy)ethane	37853-59-1	Not registered under REACH
B	DDC-CO	Dechlorane Plus	13560-89-9	Registered under REACH under 100-1000 T/y tonnage band
C	TEHP	Tris(2-ethylhexyl) phosphate	78-42-2	Registered under REACH under 1000-10000 T/y tonnage band
C	EHDPP	2-ethylhexyl diphenyl phosphate	1241-94-7	Registered under REACH under 1000-10000 T/y tonnage band
C	DDC-DBF	Dechlorane 602 (1,2,3,4,6,7,8,9,10,10,11,11-Dodecachloro-1,4,4a,5a,6,9,9a,9b-octahydro-1,4:6,9 dimethanodibenzofuran)	31107-44-5	Not registered under REACH
C	DBDPE	Decabromodiphenylethane	84852-53-9	Registered under REACH under the 10000-100000 T/y tonnage band and under CoRAP (suspected PBT/vPvB, High (aggregated) tonnage and Wide dispersive use).
C	TEP	Triethyl phosphate	78-40-0	Registered under REACH

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Cat.	Abbrev./ Acronym	Systematic name	CAS No.	Regulation
C	HBB	Hexabromobenzene	87-82-1	Not registered under REACH
C	DBE-DBCH	Tetrabromoethylcyclohexane	3322-93-8	Not registered under REACH
C	DBHCTD	Hexachlorocyclopentenyl dibromocyclooctane	51936-55-1	Not registered under REACH
C	PBEB	Pentabromoethylbenzene	85-22-3	Not registered under REACH
C	DDC-Ant	Dechlorane 603 (1,2,3,4,5,6,7,8,12,12,13,13-Dodecachloro-1,4,4a,5,8,8a,9,9a,10,10a-decahydro-1,4:5,8:9,10-trimethanoanthracene)	13560-92-4	None
C	2,4,6-TBP	2,4,6-tribromophenol	118-79-6	Not registered under REACH but under CoRAP (suspected PBT/vPvB, CRM, High (aggregated) tonnage, High RCR, Wide dispersive use)
C	PBT	Pentabromotoluene	87-83-2	Not registered under REACH
C	PBB-Acr	Pentabromobenzyl acrylate	59947-55-1	Registered under REACH under 100-1000 T/y tonnage band
C	V6	2,2-bis(chloromethyl)trimethylenebis[bis(2-chloroethyl) phosphate]	38051-10-4	Registered under REACH under the 100-1000 T/y tonnage band
D	ip-TPP	Isopropyl triphenyl phosphate	68937-41-7	Registered under REACH under the 1000-10000 T/y tonnage band
D	BPA-BDPP	Bisphenol A bis(diphenylphosphate)	5945-33-5	Registered under REACH
D	TBCO	1,2,5,6-tetrabromocyclooctane	3194-57-8	None
D	PBP	Pentabromophenol	608-71-9	Not registered under REACH
D	DBP	2,4-dibromophenol	615-58-7	Not registered under REACH
D	TIBP	Tri-iso-butyl phosphate	126-71-6	Registered under REACH under the 1000-10000 T/y tonnage band
D	TnPP	Tri-n-propyl-phosphate	513-08-6	Not registered under REACH
D	TDBPP	Tris(2,3-dibromopropyl) phosphate	126-72-7	Restricted under REACH

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Cat.	Abbrev./ Acronym	Systematic name	CAS No.	Regulation
D	CDP	Cresyl diphenyl phosphate	26444-49-5	Not registered under REACH
D	HCTBPH	Dechlorane 604 (1,2,3,4,7,7-hexachloro-5-(2,3,4,5-tetrabromophenyl)-bicyclo[2.2.1]hept-2-ene)	34571-16-9	Not registered under REACH
D	OBTMPI	Octabromotrimethylphenyl indane	1084889-51-9, 1025956-65-3, 893843-07-7, 155613-93-7	Not registered under REACH
D	TBX	2,3,5,6-tetrabromo-p-xylene	23488-38-2	Not registered under REACH
E	DBNPG	Dibromoneopentylglycol	3296-90-0	Registered under REACH under the 100-1000 T/y tonnage band
E	TDBP-TAZTO	Tris(2,3-dibromopropyl)isocyanurate	52434-90-9	Not registered under REACH
E	RBDPP	Resorcinol bis(diphenyl phosphate)	57583-54-7	Not registered under REACH
E	TTBNPP	Tris(tribromoneopentyl)phosphate	19186-97-1	Registered under REACH under the 100-1000 T/y tonnage band
E	EBTEBPI	N,N'-ethylenebis(tetrabromophthalimide)	32588-76-4	Registered under REACH under the 100-1000 T/y tonnage band and under CoRAP (suspected PBT/vPvB, consumer use, Exposure of environment, Exposure of workers, Wide dispersive use)
E	HEEHP-TEBP	2-(2-hydroxyethoxy)ethyl 2-hydroxypropyl 3,4,5,6-tetrabromophthalate	20566-35-2	Registered under REACH under 100 – 1000 T/y
E	TTBP-TAZ	2,4,6-tris(2,4,6-tribromophenoxy)-1,3,5-triazine	25713-60-4	Not registered under REACH
E		Melamine polyphosphate	20208-95-1, 218768-84-4	Not registered under REACH
E		Diethylphosphinic acid	813-76-3	Not registered under REACH
E	BDBP-TAZTO	1,3-bis(2,3-dibromopropyl)-5-(2-propen-1-yl)-1,3,5-triazine-2,4,5(1H,3H,5H)-trione	75795-16-3	None

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Cat.	Abbrev./ Acronym	Systematic name	CAS No.	Regulation
E	4'-PeBPO-BDE208	Pentabromophenoxy-nonabromodiphenyl ether	58965-66-5	Not registered under REACH
E	TBNPA	Tribromoneopentyl alcohol	1522-92-5	Registered under REACH under 100 – 1000 T/y
E	HBCYD	Hexabromocyclodecane	25495-98-1	None
E	DBS	Dibromostyrene	31780-26-4	Not registered under REACH
E	DBP-TAZTO	1-(2,3-dibromopropyl)-3,5-diallyl-1,3,5-triazine-2,4,6(1H,3H,5H)-trione	57829-89-7	None

Table 13-2: Compounds recommended to remove from priority list

Cat.	Abbrev./ Acronym	Systematic name	CAS No.	Notes
NA	Mirex/Dechlorane	Perchloropentacyclodecane	2385-85-5	Mirex was previously listed in FR target list, however it is banned under the Stockholm Convention, and has not been in use in EU for >35 years. It is recommended to be excluded from further HBM activities.

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Table 13-3: Compounds to be considered for addition to priority list

Cat.	Abbrev./ Acronym	Systematic name	CAS No.	Notes
E		benzene, ethenyl-, polymer with 1,3-butadiene, brominated	1195978-93-8	Suggested by ECHA; selected by a large part of the Expanded Polystyrene (EPS) and Extruded Polystyrene (XPS) Industry as replacement to HBCDD, suspected persistence (not registered under REACH because a polymer)
E		1,1'-(isopropylidene)bis[3,5-dibromo-4-(2,3-dibromopropoxy)benzene]	21850-44-2	Suggested by ECHA; registered under REACH under the 1000-10000 T/y tonnage band and under CoRAP (suspected PBT/vPvB, endocrine disruptor, High (aggregated) tonnage)
E		1,1'-(isopropylidene)bis[3,5-dibromo-4-(2,3-dibromo-2-methylpropoxy)benzene]	97416-84-7	Suggested by ECHA; registered under REACH under the 100-1000 T/y tonnage band and under CoRAP (suspected PBT/vPvB, endocrine disruptor, Exposure of environment)
E		bis(α,α -dimethylbenzyl) peroxide	80-43-3	Suggested by ECHA; used as a flame retardant synergist; registered under REACH under the 10000-100000 tonnage band and under CoRAP (suspected PBT/vPvB, Consumer use, Exposure of environment, Exposure of workers, High (aggregated) tonnage, High RCR, Wide dispersive use)
E		(pentabromophenyl)methyl acrylate	59447-55-1	Suggested by ECHA, registered under REACH under the 100-1000 T/y tonnage band
E		2-Butyne-1,4-diol, polymer with 2-(chloromethyl)oxirane, brominated, dehydrochlorinated, methoxylated	68441-62-3	Suggested by ECHA, registered under REACH under the 1,000-10,000 T/y tonnage band
E		2,2,6,6-tetrakis(bromomethyl)-4-oxaheptane-1,7-diol	109678-33-3	Suggested by ECHA, registered under REACH under a confidential tonnage band

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13.3 Policy related questions

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?
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)?
3. 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?
4. How does exposure to FRs differ between adults and children, males and females?
5. How does exposure differ by geographic area within Europe? Do countries/regions have different FR exposure levels?
6. Are there one or more occupationally exposed sub-groups? What occupations are associated with high exposure to FRs?
7. Is elevated exposure to FRs associated with particular consumption patterns or lifestyles?
8. What are the relevant exposure pathways for FRs, e.g., diet, air, water, indoor environmental exposure?
9. Do certain flame retardants co-occur in HBM matrices?
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?
11. Can exposure to FRs be linked with any adverse health effects?
12. What are the population groups most at risk?
13. As FR market shifts towards replacement/alternative FRs, does human exposure reflect that trend? E.g., DBDPE as replacement for BDE-209;
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?
15. Can reference values be established for any FRs?

13.4 Research activities to be undertaken

The list of FRs is extensive, and not fixed, as new FRs are identified in human and environmental matrices on a regular basis. Therefore, flexibility must be maintained in the list of relevant and priority compounds. However, of the current list of 62 FRs, we highlight 20 individual compounds to receive attention based on evidence of toxicity but a lack of HBM data.

- ▶ **TPHP, TMPP, TCEP, TCIPP, TDCIPP, TNBP, TBBPA, and TBOEP** are Cat. B compounds for which available HBM data suggests significant human exposure, and there is sufficient evidence of toxicity to warrant concern
- ▶ **TEHP, EHDPP, DDC-DBF, ip-TPP, V6, 2,4,6-TBP and TDBPP** are Cat. C and D compounds with very limited HBM data, and in some cases none at all within Europe, but suggestion of toxicological concern.
- ▶ **DBNPG, TDBP-TAZTO, RBDPP, melamine polyphosphate and EBTEBPI** are Cat. E compounds for which no HBM data exists but toxicological evidence suggests concern.

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Additionally, we highlight the 6 compounds which entirely lack toxicological and HBM data: diethylphosphinic acid, BDBP-TAZTO, 4'-PeBPO-BDE208, HBCYD, DBS and DBP-TAZTO. These compounds should receive attention in the form of suspect screening to determine if they are present in any human matrices and warrant further attention.

Table 13-4: Listing of research activities to be carried out to answer the policy questions summed up in 1.3

Policy question	Substance	Available knowledge ²⁸	Knowledge gaps and activities needed
1, 3, 4, 5, 6, 7, 8, 11, 13, 15	PBDEs (BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, BDE-183, BDE-209)	<ul style="list-style-type: none"> ▶ Established analytical methods, widely available analytical standards, reference materials ▶ Existing information on temporal trends and geographic differences in human matrices and exposure pathways (e.g.,^{26,33,55,56}) ▶ Biomonitoring data for PBDEs in a range of human matrices (primarily serum, maternal milk) in a large number of studies: <ul style="list-style-type: none"> ○ Sweden^{33,35,44,46,49,56-72} ○ Norway^{51,73-80} ○ Germany⁸¹⁻⁸³ ○ France^{31,84-87} ○ Denmark^{86,88,89} ○ Finland^{86,90,91} ○ Belgium⁹²⁻⁹⁶ ○ Netherlands⁹⁷⁻¹⁰¹ ○ Spain¹⁰²⁻¹⁰⁷ ○ Poland¹⁰⁸ ○ Austria¹⁰⁹ ○ Czech Republic¹¹⁰⁻¹¹³ ○ Italy¹¹⁴ ○ UK⁴⁷ ○ Greece^{32,115} 	<p>Gaps:</p> <ul style="list-style-type: none"> ▶ Biomonitoring data for Southern and Central/Eastern Europe ▶ Coherence and synthesis in data <p>Activities:</p> <ul style="list-style-type: none"> ▶ Synthesis and/or meta-analysis of existing HBM data to identify time trends in exposure and possible regional differences. Inform on whether current regulatory structure can effectively lead to decreases in human exposure <p>Statistical evaluation of average concentrations, time trends and potential variance between population subgroups both regional and at risk (meta-analysis).</p>

²⁸ Complete database of evaluated HBM knowledge is available upon request from flame retardants CGL

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Policy question	Substance	Available knowledge ²⁸	Knowledge gaps and activities needed
1, 3, 4, 5, 6, 7, 8, 11, 13, 15	HBCDD	<ul style="list-style-type: none"> ▶ Established analytical methods, widely available analytical standards, reference materials ▶ Biomonitoring data for HBCDDs in many studies in a range of human matrices (primarily serum, maternal milk): <ul style="list-style-type: none"> ○ Belgium^{28,92-94,96} ○ Norway^{28,51,77-80,116} ○ Netherlands⁹⁸⁻¹⁰⁰ ○ France^{84,86} ○ UK⁴⁷ ○ Denmark⁸⁶ ○ Finland⁸⁶ ○ Sweden^{35,46,49,56,60} ○ Germany⁸³ ○ Czech Republic^{111,112} ○ Spain⁵² ○ Greece³² 	<p>Gaps:</p> <ul style="list-style-type: none"> ▶ Biomonitoring data for Southern and Central/Eastern Europe ▶ Coherence and synthesis in data <p>Activities:</p> <ul style="list-style-type: none"> ▶ Synthesis and/or meta-analysis of existing HBM data needed to identify time trends in exposure and possible regional differences. Inform on whether current regulatory structure can effectively lead to decreases in human exposure <p>Statistical evaluation of average concentrations, time trends and potential variance between population subgroups both regional and at risk (meta-analysis).</p>
2, 3, 4, 5, 8, 9, 10, 11, 13	Cat. B	<p>Biomonitoring data for NBFs and CFRs in milk, serum for selected countries, small study sizes:</p> <ul style="list-style-type: none"> ○ France³¹ ○ Germany¹¹⁷ ○ Norway^{118,119} ○ Netherlands⁹⁷ ○ Sweden^{46,72,120} ○ UK^{47,121} ○ Belgium^{96,121-123} ○ Finland¹²⁴ ○ Greece¹²⁵ ○ Romania¹²⁵ ○ UK⁴⁷ ○ Ireland¹²⁶ ○ Czech Rep.¹¹² ○ Slovakia¹²⁷ ○ France^{31,84,128} <p>Many studies report only TBBPA or a sub-set of Cat. B FRs</p> <p>Biomonitoring data for OPEs, usually OPE metabolites in urine. Studies usually report a sub-set of the OPEs; methods vary widely between studies</p> <ul style="list-style-type: none"> ○ Norway^{36,118,129,130} ○ Germany^{37,131,132} ○ Finland¹²⁴ ○ Sweden¹³³⁻¹³⁶ ○ Belgium¹³⁷ 	<p>Interlaboratory validation exercises</p> <p>Development of SOPs for determination of compounds in target human matrices</p> <p>Synthesis of existing data regarding biomonitoring and exposure – evaluation of data gaps for regions and compounds.</p> <p>Screening of existing HBM projects or biobank archives for Cat. B substances with lack of HBM data. Particular data gap for Southern and Eastern Europe</p>

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Policy question	Substance	Available knowledge ²⁸	Knowledge gaps and activities needed
	Cat. C substances	<p>HBM data for individual locations, or based on small method development studies; variability in matrices and analytical methods; many values below detection limits:</p> <ul style="list-style-type: none"> ○ TEHP^{118,124,129} ○ EHDPP^{129,130,136} ○ DDC-DBF^{31,76,117,119,122} ○ DBDPE^{46,47,59,76,119,122,126,138} ○ HBB^{46,76,119,122,126,127,138} ○ DBE-DBCH^{46,47,59,127} ○ DBHCTD^{76,119,127,139} ○ PBEB^{46,59,127,138} ○ DDC-Ant^{31,76,117,119,122} ○ 2,4,6-TBP^{73,75,140} ○ PBT^{59,127} ○ PBB-Acr¹²⁷ ○ V6¹⁴¹ 	<p>Evaluation of published methods to determine validity and applicability.</p> <p>Assessment of HBM data quality – appropriateness of monitored matrices for target compounds</p> <p>Screening of existing data regarding biomonitoring and exposure for all target FR – evaluation of data gaps for regions and compounds.</p> <p>Screening of existing HBM projects or biobank archives for Cat. C substances.</p>
2, 9, 10, 14	Cat. D substances	<p>Limited HBM data, often none from Europe:</p> <ul style="list-style-type: none"> ○ OBTMPI^{139,142} ○ TIBP^{129,143} ○ TBX^{46,59,139,144} ○ TBCO¹²⁷ ○ HCTBPH^{139,145} ○ BPA-BDPP¹⁴⁶ ○ ip-TPP¹⁴⁷ ○ PBP¹⁴⁰ ○ TnPP^{148,149} 	<p>Evaluation of existing methods, matrices to provide recommendations for future screening or method development.</p> <p>Screen (semi-quantitative) for presence of compounds in human and/or environmental matrices, using existing biobank archives where possible</p> <p>Develop validated methods to improve quantification for compounds that are consistently identified or listed as high concern based on gathered toxicity information</p>
2, 14	Cat. E substances	<p>No available HBM or toxicity information for diethylphosphinic acid, BDBP-TAZTO, 4'-PeBPO-BDE208, HBCYD, DBS and DBP-TAZTO</p> <p>Toxicity information but no HBM data for DBNPG, TDBP-TAZTO, RBDPP, TTBNPP, EBTEBPI, HEHP-TEBP, TTBP-TAZ, and melamine polyphosphate</p>	<p>Screen (semi-quantitative) for presence of compounds in human and/or environmental matrices, using existing biobank archives where possible</p> <p>Develop validated methods to improve quantification for compounds that are consistently identified or listed as high concern based on gathered toxicity information</p>

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14 Prioritised substance group: Lead

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14.1 Background information

14.1.1 Hazardous properties

Lead, a silvery grey metal, has some unique properties, like soft softness, high malleability, low melting point, ductility and resistance to corrosion, which contributed to its widespread use. It exists in different forms (elemental, inorganic and organic) which have different chemical and toxicological properties.

Lead is found in concentrated and easily accessible lead ore deposits that are widely distributed throughout the world. Inorganic lead compounds exist in two oxidative states: +2 and +4. The former one is more common. Lead also forms stable organic compounds (e.g., as tetraethyl lead and tetramethyl lead). Water solubility of lead compounds varies widely: lead sulphides and lead oxides are purely soluble, while nitrate, chlorate and chloride salts are reasonably soluble in cold water. Organolead compounds are highly lipophilic and characterised by low water solubility.

Lead has been classified by the German Research Foundation (MAK Commission) in category 2, to be regarded as human carcinogen. IARC classified lead (in general) as possibly carcinogenic to humans (Group 2B) (IARC, 1987), inorganic lead compounds as probably carcinogenic to humans (Group 2A) (IARC, 2006); however, the evidence for organic lead compounds was considered to be inadequate in humans and animals (Group 3) (IARC, 2006).

14.1.1.1 Absorption, distribution, metabolism and excretion

Gastrointestinal absorption of ingested lead is influenced by physiological factors (e.g. age, fasting, nutritional calcium and iron status, pregnancy) and the physicochemical characteristics of particles (size, solubility, and lead species). The extent and rate of absorption are also influenced by the ingested dose (Jakubowski, 2012).

Deposition and absorption of inhaled lead-containing particles are influenced by their size and solubility. Large particles are transferred by mucociliary transport into the pharynx and then swallowed, with possible absorption from the gastrointestinal tract. Smaller particles can be deposited in the alveolar part of the lungs and almost completely absorbed after extracellular dissolution or ingestion by phagocytic cells (Bailey and Roy 1994).

Dermal absorption of inorganic lead compounds is generally considered to be much less than absorption by inhalation or oral routes of exposure. In contrast, animal studies have showed that organic lead compounds are rapidly and extensively absorbed through the skin (ATSDR, 2019).

The distribution of lead in the body is independent of the exposure route. Lead in blood is found primarily in the red blood cells (96-99%). The half-life of lead in blood is approximately 30 days in adult male humans but it varies depending on the level of exposure, sex and age (Jakubowski, 2012).

Half-life of lead in bones is approximately 10-30 years (EFSA, 2010), but it can be mobilised by certain physiological processes like pregnancy or other factors. Lead can be transferred from the mother to the fetus and also from the mother to infants via maternal milk (ATSDR, 2019).

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Inorganic lead forms complexes with a variety of protein and nonprotein ligands (e.g., albumen, nonprotein sulfhydryls as extracellular ligands or ALAD as intracellular ligand), while alkyl lead compounds are actively metabolised in the liver by oxidative dealkylation catalysed by cytochrome P-450 (ATSDR, 2019).

Lead is excreted primarily in urine and feces independent of the route of exposure; sweat, saliva, hair and nails, breast milk and seminal fluids are minor routes of excretion (ATSDR, 2019).

14.1.1.2 Health effects

14.1.1.2.1.1 General overview of health effects

Based on the estimation of the Institute for Health Metrics and Evaluation (IHME), lead exposure accounted for 1.06 million deaths and 24.4 million years of healthy life lost (disability-adjusted life years (DALYs) worldwide in 2017 due to long-term effects on health. The highest burden was present in low- and middle-income countries. IHME also estimated that in 2016, lead exposure accounted for 63.2% of the global burden of idiopathic developmental intellectual disability, 10.3% of the global burden of hypertensive heart disease, 5.6% of the global burden of the ischaemic heart disease and 6.2% of the global burden of stroke (IHME, 2017). Lead is associated with a wide range of toxicity in children. These toxic effects extend from acute, clinically obvious, symptomatic poisoning at high levels of exposure down to subclinical (but still very damaging) effects at lower levels. Lead poisoning can affect virtually every organ system in the body. The principal organs affected are the **central and peripheral nervous system** and the cardiovascular, gastrointestinal, renal, endocrine, immune and haematological systems. (WHO, 2010).

14.1.1.2.1.2 Acute clinical toxicity

Intense, acute, high-dose exposure to lead can cause symptomatic poisoning in children. It is characterised by colic, constipation, fatigue, anaemia and neurological features that can vary from poor concentration to stupor. In the most severe cases, a potentially fatal acute encephalopathy with ataxia, coma and convulsions can occur. In many instances, children who survive acute lead poisoning go on to have permanent and clinically apparent deficits in their neurodevelopmental function (Byers & Lord, 1943, cit in WHO, 2010).

14.1.1.2.1.3 Subclinical (chronic) toxicity

The subclinical toxic effects of lead can be very damaging. The premise underlying the concept of subclinical toxicity is that there is a dose-related continuum of toxic effects in which clinically apparent effects have their asymptomatic (but still very real) counterparts (Landrigan, 1989).

Haematological toxicity

Due to blocking the enzymes involved in heme synthesis (δ -aminolevulinic acid dehydratase (δ -ALAD), ferrochelatase) and oxidative damage of erythrocyte membranes anaemia is the classic clinical manifestation of lead toxicity in erythrocytes (Schwartz et al., 1990; EHC, 1995). Therefore, ALAD is being used as a biomarker for testing Pb toxicity. The severity and prevalence of lead-induced anaemia correlate directly with the blood lead concentration. Younger and iron deficient children are at higher risk of lead-induced clinical anaemia.

The anaemia induced by lead is caused primarily by impairment of the haem biosynthesis, but an increased rate of erythrocyte destruction may also occur (Schwartz et al., 1990).

Reproductive toxicity

Lead exposure may damage fertility, may damage the unborn child (reduced foetal growth and disturbed maturation, pre-term delivery) and may cause harm to breast-fed children (Sun et al.,

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2019). Lead can easily cross the placental barrier, therefore can readily enter the bloodstream of the foetus. Since lead can also pass the blood brain barrier, neurological development is of great concern when prenatal exposure to lead occurs (Baeyens et al., 2014).

Neurotoxicity

Neurodevelopmental effect of lead is the most important hazard of chronic lead exposure from public health point of view. In the central nervous system, lead causes asymptomatic impairment of neurobehavioural function in children at doses insufficient to produce clinical encephalopathy. The dose–response relationship between blood lead levels and loss of IQ was found to be stronger at blood lead levels lower than 10 µg/dl than at higher levels (Lanphear et al., 2000). An international pooled analysis of data from seven cohorts has confirmed these findings (Lanphear et al., 2005)

An increase in blood lead level from less than 1 µg/dL to 10 µg/dL was associated with a six IQ point decrement, which is considerably greater than the decrement associated with an increase in blood lead level from 10 µg/dL to 20 µg/dL. The findings of this pooled analysis – that there are adverse effects below 10 µg/dL and that the effects are steepest at the lowest levels of exposure – have been confirmed by numerous investigators (Emory et al., 1999, 2003; Bellinger & Needleman, 2003; Wasserman et al., 2003; Chiodo, Jacobson & Jacobson, 2004; Despres et al., 2005; Fraser, Muckle & Despres, 2006; Hu et al., 2006; Kordas et al., 2006; Schnaas et al., 2006; Tellez-Rojo et al., 2006; Chiodo et al., 2007; Surkan et al., 2007, all cit. in WHO, 2010). Recent studies confirmed previous data on long-term lead exposure caused reduction in intellectual functioning and hippocampal-dependent memory and learning dysfunction. School-age children with higher blood lead level have poor long-term memory ability (Zeng et al., 2019). In a recent pre-birth prospective cohort with maternal lead levels below 5 µg/dL, there have been found a trend of worse neurobehavioral scores with increasing prenatal lead concentrations, in particular for mid-childhood emotional problems and capacity to plan/organise and shift (Fruh et al., 2019). In a Polish cohort study, fetal exposure to very low lead levels (0.99 ± 0.15 µg/dL in maternal blood and 0.96 ± 0.16 µg/dL in the cord blood) was found to affect early cognitive function, with boys being more susceptible than girls (Polanska et al., 2018). The greater susceptibility of boys was also reported in a Canadian cohort, where prenatal blood lead concentrations below 5 µg/dL were still associated with a decline in cognitive function in, but only for boys (Desrochers-Couture, 2018).

When a population's exposure to lead is sufficiently widespread to cause a decrease in its mean IQ, there results a substantial increase in the number of children with diminished intelligence and mental retardation. At the same time, there is a substantial reduction in the number of children with truly superior intelligence. The consequences are: (a) a substantial increase in the number of children who do poorly in school, who may require special education and other remedial programmes, and who may not contribute fully to society when they become adults; (b) a reduction in a country's future leadership; and (c) a widening gap in socioeconomic attainment between countries with high and low levels of population exposed to lead (Needleman et al., 1979).

However, adverse effects of chronic lead exposure on cognitive function were observed not only in children. Sufficient evidence exists to conclude that there is an association between lead dose and decrements in cognitive function in adults, too. Overall, while the association between blood lead levels and cognitive function is more pronounced in occupational groups with high current lead exposures, associations between bone lead levels and cognitive function are more evident in studies of older subjects with lower current blood lead levels, particularly in longitudinal studies of cognitive decline. (Shih RA et al., 2007). A recent meta-analysis also concluded, that neurocognitive performance in adults with occupational or environmental lead exposure was significantly impaired. Based on a marginally significant ($p=.06$) effect of difference in exposure

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levels, a blood lead increase of 10 µg/dL translated into a decline in cognitive abilities of Hedges $g = .09$ (Vlasak et al., 2019).

Cardiovascular toxicity

An increasing body of evidence suggests an association between low-level lead exposure and clinical cardiovascular outcomes and cardiovascular mortality (Lanphear et al., 2018; Harari et al., 2019). There is a link between blood lead level and increase of blood pressure in pregnant women at low level exposure (Wells et al., 2011). Maternal prenatal toenail Pb was associated with statistically significant increases in child systemic blood pressure (Farzan et al., 2018). Bone lead was also associated with elevated systolic blood pressure in lead exposed workers (Barry et al., 2019). A previous systematic review evaluating the evidence on the associations between lead exposure and cardiovascular endpoints in human populations concluded that the evidence is sufficient to infer a causal relationship of lead exposure with hypertension (Navas-Acien et al., 2007).

Cancer risk

Epidemiological studies indicate higher risk of cancers of the stomach, lung, kidney, and brain in workers exposed to inorganic lead (Steenland, 2019, Barry et al, 2019). Several studies indicated its genotoxicity and ability to generate reactive oxygen species. (IARC, 2014). IARC classified inorganic lead compounds as probably carcinogenic to humans (Group 2A) (IARC, 2006).

14.1.1.2.1.4 Genetic and epigenetic factors influencing lead toxicity

Oxidative stress caused by lead exposure

Data from the literature show the involvement of oxidative stress in the mechanism of lead toxicity (Lopes et al., 2016). Lead causes oxidative stress of membranes and depletion of body antioxidants. Maternal blood Pb was associated with increased cord blood mtDNA content, a marker of oxidative stress (Sanchez-Guerra, 2019). Oxidative stress index was higher in workers with occupational exposure to lead (Qu et al., 2019).

Susceptibility to lead toxicity

Some polymorphic genes (are delta-aminolevulinic acid dehydratase (ALAD) gene, vitamin D receptor (VDR), glutathione S-transferase (GST), hemochromatosis gene) were identified to be able to potentially influence the bioaccumulation and toxicity of lead in humans (Mani et al., 2019).

Epigenetic alterations related to lead exposure

Prenatal low-level lead exposure was associated with DNA methylation changes in cord blood (Wu et al, 2017). Altered epigenetic regulation (DNA methylation at specific genes and also at the global genome level, histone modifications, miRNAs) can cause neurotoxic (cognitive dysfunction, memory loss, behavioral disorders, attention deficit hyperactivity disorder, autism spectrum disorder, Alzheimer's disease) and other toxic outcomes, such as metabolic disorder, cardiovascular disorders, hematopoietic disorder, and reproductive impairment (Khalid et al., 2019). In a recent work, genetic and epigenetic biomarkers of Pb exposure, susceptibility, and effect were reviewed (Mani et al, 2019). These data may help uncover the mechanism of action and in the identification of susceptible groups.

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14.1.2 Exposure characteristics

14.1.2.1 Lead production and consumption

Lead is manufactured and/or imported in the European Economic Area in 1,000,000 – 10,000,000 tons per year (ECHA, 2018). About 50 nations mine lead in quantities ranging from a few hundred tons to more than half a million tons (U.S. Bureau of Mines, 1993). Roughly 20 nations produce only secondary (i.e., recycled) lead. Secondary smelting (recycling) of lead from lead-acid batteries from vehicles and industries has become increasingly important and by the end of the 20th century accounted for almost half of world refined lead production. Other uses of lead include pigments and other compounds, rust inhibitors, rolled and extruded products, cable sheathing, alloys, radiation shielding, ceramic glazes, plastic stabilizers, jewellery making, soldering, crystal products, fishing weights, shot and ammunition, electronic waste, use in water pipes, and fuel additives (The Global Dimensions of Lead Poisoning: An Initial Analysis, 1994). Due to regulation in Europe on the use of lead in dyes and ceramics it is expected that exposure through these applications is decreasing. Global consumption of lead is increasing today, because of increasing demand for energy-efficient vehicles. The largest current use of lead is in storage batteries for cars and other vehicles. (WHO, 2010).

14.1.2.2 Lead exposure routes

Although some exposure to lead results from direct contact with lead containing products, human exposure more frequently occurs via environmental media such as air, water, and soil. Based on worldwide collection of results of airborne lead concentrations measured before 1994, it was concluded that lead levels in both air and soil were generally higher in urban areas and near industrial sources than in other areas (median values in urban areas were 1.075 µg/m³, in suburban ones 0.33 µg/m³ and in rural areas 0.1 µg/m³). In urban areas, air and soil levels were associated with use of leaded petrol. Lead concentrations in both air and soil increased with traffic density and proximity to roads, as well as with higher lead concentrations in petrol. (The Global Dimensions of Lead Poisoning: An Initial Analysis, 1994).

The ECHA (2018) is mentioning that releases of lead to the environment is likely to occur from:

- ▶ outdoor use in long-life materials with low release rate (e.g. metal, wooden and plastic construction and building materials)
- ▶ indoor use in long-life materials with low release rate (e.g. flooring, furniture, toys, construction materials, paints, curtains, foot-wear, leather products, paper and cardboard products, electronic equipments)
- ▶ indoor use in close systems with minimal release (e.g. cooling liquids in refrigerators, oil-based electric heaters)
- ▶ outdoor use in close systems with minimal release (e.g. hydraulic liquids in automotive suspension, lubricants in motor oil and break fluids)

Human exposure to lead from drinking water results primarily from lead leaching from leaded plumbing components, rather than contamination of source waters (i.e., lakes, rivers, and aquifers).

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The following sources and products account for most cases of childhood exposure to lead and lead poisoning (WHO, 2010):

- ▶ lead from an active industry, such as mining (especially in soils),
- ▶ lead-based paints and pigments,
- ▶ lead solder in food cans,
- ▶ ceramic glazes,
- ▶ drinking-water systems with lead solder and lead pipes,
- ▶ lead in products, such as herbal and traditional medicines, folk remedies, cosmetics and toys,
- ▶ lead released by incineration of lead-containing waste,
- ▶ lead in electronic waste (e-waste),
- ▶ lead in the food chain, via contaminated soil,
- ▶ lead contamination as a legacy of historical contamination from former industrial sites.

Human exposure routes:

- ▶ **Inhalation:** inhalation of lead particles generated by burning materials containing lead (e.g. during smelting, recycling, stripping leaded paint, and using leaded petrol or leaded aviation fuel),
- ▶ **Oral:** ingestion of lead-contaminated dust, water (from leaded pipes), food from lead-glazed or lead-soldered containers, highly consumed food with low/medium lead content (e.g. grains) or food with known elevated lead content (e.g. mussels and lead-shot game meat),
- ▶ **Trans placental:** lead in bone is released into blood during pregnancy and becomes a source of exposure to the developing fetus. Moreover, lead is transmitted by maternal milk to infants.

14.1.2.3 Availability of HBM data

Surveys measuring blood lead levels in the general population have been conducted in several countries since the early 1980. After phasing out lead from petrol in most of the European countries interest in blood lead levels has been faded for a while. Results of blood lead level surveys conducted during the past two decades among the general population were found to be available in sixteen European countries (see Table 1), most of them covered children population, too. Decreasing trend in blood lead level of children could be observed with lowering lead content of petrol and finally phasing out leaded petrol in various countries. However, e.g. in Sweden it was found that after 2009 the decrease in the blood lead level discontinued (Wennberg et al., 2017) which means that there are still other existing lead exposure sources to be detected and eliminated.

Unfortunately, there are very few data on the present blood lead levels among the general population in the European countries. In an intensive literature search only 8 countries (Belgium, Germany, Denmark, Kosovo, Poland, Spain, Slovenia and Sweden) were found from where blood lead levels measured during the past 5 years were available.

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Table 14-1: Summary of European blood lead surveys reported in the past 10 years

Country	Study	Population studied	N	Year of sampling	PbB (µg/L)	Reference
Armenia	3 towns adjacent to metal mining and smelting industries	4 – 6 years	159	2013	GM: 60.0 S.D.: ± 30.0	Grigoryan et al. (2016)
Belgium	FLEHS I	newborns	1,072	2002-2006	GM: 13.7; 95% C.I.:12.9-14.6	Schoeters et al. (2017)
		adolescents	1,650	2002-2006	GM: 22.5; 95% C.I.:21.8-23.3	
	FLEHS II	newborns	241	2007-2011	GM: 8.6; 95% C.I.:8.0-9.2	
		adolescents	207	2007-2011	GM: 14.6; 95% C.I.:13.8-15.5	
	FLEHS III	newborns	281	2012-2015	GM: 6.4; 95% C.I.:6.0-6.7	Fierens et al. (2016)
		adolescents	204	2012-2015	GM: 9.5; 95% C.I.:9.0-10.0	
	Ath (Hainaut province)	2.5- 6 years	98	2009	GM: 16.6; 95% C.I.:14.8-18.2	
		7-11 years	74	2009	GM: 14.8; 95% C.I.:13.2-16.6	
40-60 years men		52	2009	GM: 31.7; 95% C.I.:27.9-36.1		
40-60 years women		54	2009	GM: 21.4; 95% C.I.:18.1-25.3		
Croatia	Koprivnica	7-14 years	46	2007-2008	GM: 17.9; Range:10.0-42.0	Hrubá et al. (2012)
Czech Republic	Prague	7-14 years	8	2007-2008	GM: 15.5; Range:12.0-22.0	Hrubá et al. (2012)
	CZ-HBM	18-58 years	4,472	1994-2003 and 2005-2009	GM: 23.0	Cerna et al. (2012)
		8-10 years	3,798		GM: Boys: 22.0; Girls: 19.0	
		breastfeeding primipare	5,667		GM: 14.0	
Denmark	Snart Forældre/Milieu	18-40 years women	73	2011-2014	GM: 8.1 (95th% 15.8)	Rosofsky et al. (2017)
Finland	NFBC	31 years males	126	1997	GM: 17.06 S.D.:± 1.84	Abass et al. (2017)
		31 years females	123	1997	GM: 9.06; S.D.: ± 2.20	

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Country	Study	Population studied	N	Year of sampling	PbB ($\mu\text{g/L}$)	Reference
France	ENNS 2006-2007	18-39 years	579	2006-2007	GM: 19; 95%C.I.: 44-62	Falq et al. (2008)
		40-59 years	947		GM: 29; 95%C.I.: 66-85	
		60-75 years	423		GM: 39; 95%C.I.: 86-115	
		Total 18-75 years	1,949		GM: 26; 95%C.I.: 68-77	
	hospital-based	1-6 years	3,831	2008-2009	GM: 14.9 (95% C.I.:14.5-15.4)	Etchevers et al. (2014)
Germany	GerES I	adults	2,731	1985-1986	GM: 61	Schulz et al. (2017)
	GerES II	adults	4,287	1990-1992	GM: 45	
		children	812	1990-1992	GM: 32	
	GerES III	adults	4,822	1997-1999	GM: 32	
	GerES IV	3-14 years	1,790	2003-2006	GM: 17	
	GerES V	3 – 17 years	2,500	2014-2017	not yet available	
Hungary	NKFP (past hot spots)	4 – 15 years	253	2006	GM: 30	Rudnai et al. (2009)
Italy	PROBE	18-65 years	1,423	2008-2011	GM: 19.9 (95% C.I.:19.2-20.5)	Bocca et al (2013)
Kosovo	Mitrovica	5-11 years	166	? 2012-2014	AM: 24 \pm 19 (Range: 5-163)	Kutllovci-Zogaj et al (2014)
	Shtime (control)	6-12 years	53	? 2012-2014	AM: 23 \pm 7 (Range: 12-52)	
	Mitrovica	kindergarten	31	? 2012-2014	AM: 38 \pm 13 (Range: 22-77)	
Poland	Upper Silesia	3-18 years	4,882	1999-2013	? (not available in abstract)	Pelc et al. (2016)
	REPRO_PL	pregnant women	594	2007-2011	GM: 11.0; Range: 3.0-57.0	Polanska et al (2014)
	Szczecin	2-18 year	78	? 2010-2011	AM: 19.7 \pm 13.59	Szkup-Jabłońska et al. (2012)
	Piekary Śląskie (Silesia)	3 – 6 year	678	2013	GM: 24.7 \pm 17.5	Kowalska et al (2018)
Slovakia	Banska Bystrica	7-14 years	57	2007-2008	GM: 19.4; Range: 8.0-47.0	Hruba et al. (2012)

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Country	Study	Population studied	N	Year of sampling	PbB ($\mu\text{g/L}$)	Reference
Slovenia	Ljubljana	7-14 years	42	2007-2008	GM: 13.4; Range: 6.9-24.0	Hruba et al. (2012)
	National HBM Programme	6-11 years	174	2011 - 2014	GM: 16.1	Tratnik et al. (2013)
		men (20-35 years)	147		GM: 19.6	
		women (20-35 yrs)	127		GM: 17.3	
		women (50-60 yrs)	66		GM: 26.7	
Spain	BIOAMBIENT.ES	18-65 years	1,880	2007-2010	GM: 24 (95% CI:23.0-25.1)	Canas et al (2014)
	HEALS study	pregnant women	53	2016-2017	1 st trimester: 10, delivery: 12 cord blood: 7.9	Bocca et al (2019)
Sweden	Landskrona	7-14 years	41	2007-2008	GM: 14.0; Range: 6.0-25.0	Hruba et al. (2012)
	MONICA	adult men	619	2004-2014	25-35 yrs:11.1; 50-60 yrs:15.1	Wennberg et al (2017)
		adult women	926		25-35 yrs:9.69; 50-60 yrs:13.1	

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14.1.2.4 Guidance values

Similar guidance values were considered safe for children and adults then CDC introduced an intervention level of **25 µg/dL** for children. After recognising the special susceptibility of children to lead's toxic effects CDC formulated **10 µg/dL** as the "**value of concern**" for children in 1991 (CDC, 1991), saying that there was enough information identifying harmful effects of lead in children at blood lead levels at least as low as 10 µg/dL. At that time CDC also stated that "as yet **no threshold has been identified** for the harmful effects of lead". In 2012 CDC threw away the "value of concern" expression and decided to use a childhood BLL **reference value** of **5 µg/dL** based on the 97.5th percentile of the population BLL in children aged 1-5 to identify *children and environments associated with lead-exposure hazards* (CDC, 2012)

Epidemiological studies have provided a lot of evidence that **there is no safe level of blood lead** concentration. In Germany the German HBM Commission concluded that any setting of an "effect threshold" for blood lead levels would be arbitrary and therefore unjustified, therefore it suspended the HBM-I and HBM-II guideline values for blood lead levels in children and adults (Wilhelm et al, 2010) .

The Panel on Contaminants in the Food Chain (CONTAM Panel) of the European Food Safety Authority (EFSA) identified developmental neurotoxicity in young children and cardiovascular effects and nephrotoxicity in adults as the critical effects for the risk assessment and derived Benchmark Dose Levels (BMDLs) from blood lead levels for these effects as follows: 12 µg/L in the case of developmental neurotoxicity, 15 µg/L for chronic kidney disease and 36 µg/dL for elevated systolic blood pressure (EFSA, 2010).

There is a need for a harmonised European biological guidance value !

14.1.3 Policy relevance

14.1.3.1 Existing regulations

The EU's Drinking Water Directive (98/83/EC) aims at protection of human health from adverse effects of any contamination of water intended for human consumption. It defines the health limit value of lead in drinking water as 10 µg/L.

According to the „Proposal for a DIRECTIVE OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL on the quality of water intended for human consumption" the Commission proposes lowering the value to 5 µg/L 10 years after the entry into force of the Directive. During this transitional 10-year period, the current value of 10 µg/L will be maintained.(EU, 2017)

The 2013/39/EU Directive amending directives 2000/60/EC and 2008/1056EC as regards priority substances in the field of water policy, suggests to have lead concentration lowered to a limit of 1.2 µg/L in **inland surface water**, and 1.3 µg/L in outland surface water.

Directive 2008/50/EC of the European Parliament and of the Council sets a regulatory **limit value for lead in air** as 0.5 µg/m³ per calendar year.

Regulatory **limit value of lead in soil**: 50 – 300 mg/kg, in sludge for agriculture: 750 – 1200 mg/kg ("EUR-Lex (86/278/EEC)")

1881/2006/EC set maximum levels for certain contaminants, including **lead in foodstuffs**.

However, the Panel on Contaminants in the Food Chain (CONTAM Panel) of the European Food Safety Authority (EFSA) concluded that the present PTWI of 25 µg/kg b.w. is no longer appropriate and noted that there was no evidence for a threshold for a number of critical endpoints including

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developmental neurotoxicity and renal effects in adults. Therefore, a margin of exposure approach was applied to risk characterisation (EFSA, 2010).

Occupational exposure is regulated by the Chemical Agents Directive 98/24/EC containing both a binding OEL and a Biological Limit Value for inorganic lead and its compounds, this latter being 70 µg/dL.

14.1.4 Technical aspects

To prevent false-positive results, stringent procedures are necessary to reduce environmental contamination of blood collection devices and supplies. Consequently, venous blood collected using evacuated tubes and needles certified as “lead-free” is considered the most appropriate specimen for blood lead measurements. However, collection of venous blood from paediatric subjects is sometimes difficult; thus, capillary blood from a finger puncture is used widely for screening purposes. Published studies have compared the quality of blood lead results for capillary and venous specimens drawn simultaneously (Schlenker et al., 1994; Schonfeld et al., 1994; Parsons et al., 1997). With stringent precautions, particularly rigorous hand washing, contamination errors can be held to <4% (Parsons et al. 1997). Therefore, although venous blood is preferable for epidemiologic studies of environmental lead exposure, use of capillary blood is acceptable if collected by staff specially trained in the technique using devices certified as “lead-free.” Data should be provided showing an acceptably low rate of contamination errors and low mean bias in the capillary BLLs as collected using the study protocol (CDC, 2005).

Acceptable analytic techniques include graphite furnace atomic absorption spectroscopy (GFAAS, also known as electrothermal AAS), anodic stripping voltammetry (ASV), inductively coupled plasma atomic emission spectroscopy (ICP-AES), inductively coupled plasma mass spectrometry (ICP-MS). Information on laboratory performance (i.e., accuracy and precision) from external and internal quality control data should be provided.

14.1.5 Societal concern

Blood lead levels vary widely from country to country and region to region. The highest blood lead levels and the largest burden of disease from exposures to lead are seen in low-income countries – in particular, in areas where there are industrial uses of lead (such as smelters, mines and refineries) and/or where leaded petrol is still used heavily.

Although lead can affect children from every socioeconomic stratum, socially and economically deprived children and children in low-income countries carry the greatest burden of disease due to lead. Poor people are more likely to be exposed to lead and to be at risk of exposure to multiple sources. They are more likely to dwell on marginal land (near landfills and polluted sites), to live in substandard housing with ageing and deteriorating lead-based paint, and to live near industry, sites where waste is burned and heavy traffic. Also, lead smelting is used by marginalised populations to generate resources (WHO,2010).

The economic costs associated with childhood exposure to lead are substantial (Landrigan et al., 2002). The costs of childhood lead poisoning may be divided into *direct* and *indirect* costs. The direct or medical costs include those costs associated with the provision of medical care to children with acute lead poisoning, as well as the costs of treating cardiovascular disease in adults who have developed hypertension following exposure to lead.

Analyses of the indirect (non-medical) costs of lead poisoning have focused mainly on the loss of intelligence that is caused by lead and on the lifelong decrements in economic productivity that result from this loss of intelligence. These costs are sometimes referred to as *lost opportunity costs*. Using a conservative estimate, the decrease in intelligence attributable to each 1 µg/dl

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increase in blood lead level is 0.25 IQ points, and the decrement in lifetime economic productivity associated with each lost IQ point is 2.4%. (WHO, 2010)

14.2 Categorisation of Substances

Table 14-2: Substances included in the substance group, listed according to availability of toxicology and human biomarker data, in category A, B, C,D,E substances (see general introduction)

Category	Abbreviation/ Acronym	Systematic name	CAS No.	Regulation
A	Pb	Lead, Plumbane	7439-92-1	Regulation (EC) No 2006/1881 (food) EU 2017/738 (toys) 98/83/EC (drinking water) 2013/39/EU (surface water) 2008/50/EC (air) 86/278/EEC (soil) 98/24/EC (occupational exposure)

14.3 Policy-related questions

1. What is the concentration of lead in the human blood nowadays (after phasing out leaded petrol) in the countries of Europe?
2. Do blood lead levels of both adults and children still indicate permanent existence of lead exposure?
3. What are the sources of still existing lead exposure in different countries of Europe?
4. What kind of exposure sources are the most important for the children of various age groups and the younger or older adult population?
5. Taking the hazard from transplacental lead exposure of the unborn child into consideration, what are the blood lead levels of pregnant women?
6. Taking the presumably low concentration of lead in blood, is it feasible to measure blood lead levels in children from as small amount of blood as it can be gained from capillary samples? What criteria should be applied in order to avoid contamination from outside sources?
7. As it is difficult to connect later outcomes with exposures, which biomarkers of effects can be used in relation to effects caused by lead exposure?

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14.4 Research Activities to be undertaken

While completing this table please think of data and gaps concerning toxicology (and exposure [in three dimensions: **location** (differences between the countries), **time** (trends) and **age** (data available for which age group)]). If no HBM method is available or the method has to be harmonised within partner countries, please indicate this too.

Table 14-3: Listing of research activities to be carried out to answer the policy questions

Policy question	Substance	Available knowledge	Knowledge gaps and activities needed
1, 2	Lead	After phasing out leaded petrol, blood lead levels significantly dropped but not at the same extent and not at the same time in different countries.	Collection of information on the time and extent of phasing out lead from petrol in the various countries. Collection, comparison and evaluation of existing data on current blood lead levels and their integration into IPCheM
3,4,5	Lead	Leaded petrol used to have dominant role in blood lead levels. After its phasing out, several possible lead sources earlier thought to be insignificant (e.g. drinking water from leaded pipes, lead-containing products, etc.) may have become important, because there is no safe level of lead exposure	In order to eliminate still existing lead sources in countries <u>showing interest in participation</u> , we have to identify their importance in the exposure of different population subgroups (e.g. children 1-3 years, 4-6 yrs, 7-14 yrs and 15-18 yrs, as well as adults (19-40 years; 41-65 years; > 65 years). Special attention should be paid to pregnant women, they should be a separate group in the survey.
6	Lead	It is unquestionable, that blood lead level is the most reliable marker of lead exposure, especially in children. (In adults, bone lead content can also be used to determine lead content accumulated in the organism). Taking venous blood samples from children lacking any clinical symptoms or environment suspicious for lead contamination, only for screening purposes raises ethical concerns. Therefore, more practicable way of sampling would be capillary blood collection. In principle it is possible to use not only venous but also capillary blood samples for the determination of blood lead level but there is a risk of contamination which may obscure the very low concentrations.	In order to demonstrate availability of appropriately trained personnel, parallel measurements of blood lead levels should be performed from capillary and venous blood samples <u>in small groups of children</u> . Detailed description of sampling circumstances should be provided..

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Policy question	Substance	Available knowledge	Knowledge gaps and activities needed
7	Lead	Some biomarkers have already been identified as potential indicators for health effects associated with lead exposure; however, there are knowledge gaps.	Biomarkers of effect associated with lead exposure and health outcomes (e.g., neurodevelopment) should be reviewed to identify the most suitable indicators and to provide the baseline for further research directions.

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15 Prioritised substance group: Mercury and its organic compounds

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15.1 Background information

Mercury is a highly toxic heavy metal that poses a significant global threat to human health and the environment. Together with its various compounds, it can cause severe impacts on human health, including irreversible damage to the central nervous system. Effects can be seen even at very low levels. Fetuses, newborn babies and children are amongst the most vulnerable and sensitive to the adverse effects of mercury. Once released into the environment, mercury can move around the globe, impacting human health and environment even in remote locations, and can remain in circulation for thousands of years. Mercury in water bodies presents the greatest risk to humans since it gets converted by microorganisms into methylmercury, which is very toxic, easily absorbed by animals and bioaccumulates in the food chain. No country can control transboundary effects of mercury alone and therefore international cooperation necessary. The Minamata Convention on Mercury, which came into force in 2017, shows the global commitment to address mercury pollution. This international treaty was ratified by the European Union. Stringent European legislation is in place to restrict mercury pollution and human exposure. Although new releases to the environment in the European Union are on decline as a result of European policies, Europeans are still exposed primarily to legacy mercury and to mercury originating from sources outside the Union.

This scoping document focuses on mercury and methylmercury, the organic form of mercury, which poses the greatest risk for human health. It does not focus on inorganic forms of mercury, to which people may be exposed in the workplace.

15.1.1 Hazardous properties

15.1.1.1 Current understanding

Mercury is a naturally occurring metal in the earth's crust. It is ubiquitous in the global environment and occurs from both natural and anthropogenic sources. It exists in three main forms, which are not equally harmful: elemental (metallic), inorganic, and organic.

Elemental mercury (Hg, CAS number: 7439-97-6, EC number: 231-106-7) is a heavy, shiny, silver-white liquid. It is the only metal that is liquid at room temperature and for this reason, it is also known as "quicksilver" (European Environment Agency, 2018). It is obtained primarily from the refining of mercuric sulfide in cinnabar ore. If it is not contained, mercury vaporizes easily at room temperature to an invisible, odorless toxic gas referred to as elemental mercury vapor (Agency for Toxic Substances and Disease Registry (ATSDR), U.S. Department of Health and Human

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Services, Public Health Service, 1999). Elemental mercury is commonly used in human activities. It has been used in electrical equipment (e.g., thermostats and switches), electrical lamps, medical and laboratory equipment (e.g. thermometers, sphygmomanometers, barometers) and dental amalgams. It has also been used industrially in the production of chlorine gas and caustic soda. The anthropogenic use of mercury results into the release of large amounts into the atmosphere and can travel long distances, presenting a significant risk to human health and environment. Elemental mercury can eventually react in the atmosphere to form inorganic mercury, which gets deposited in water bodies and on land.

Inorganic mercury compounds are formed when mercury combines with other elements such as chlorine, sulfur or oxygen. Inorganic mercury compounds exist in two oxidative states, mercurous (+1) and mercuric (+2). Mercury salts are highly toxic and corrosive. Inorganic mercury compounds, such as mercuric oxide, are used in the production of batteries, polyvinylchloride, and pigments (Agency for Toxic Substances and Disease Registry (ATSDR), U.S. Department of Health and Human Services, Public Health Service, 1999).

Organic mercury compounds are formed when inorganic mercury is methylated or combines with organic agents. Different forms of organic mercury have different properties and toxicities. The most important organic form of mercury, with regards to human exposure and adverse effects on health, is methylmercury. Methylmercury is formed by anaerobic methylation of inorganic mercury by microorganisms in sediments. In waterbodies, methylmercury accumulates in aquatic organisms and biomagnifies up the food chain. The primary source of human exposure to mercury is through the consumption of fish and shellfish containing methylmercury.

Other organic mercury compounds have been used in fungicides, antiseptics and disinfectants, but have mostly been discontinued. Ethylmercury (thiomersal), is used in very small amounts in vaccines (as preservative) and pharmaceuticals. Ethylmercury is broken down by the body quickly and does not accumulate. The World Health Organization monitors and evaluates scientific evidence on the use of thiomersal as a vaccine preservative, and consistently concludes that there is no evidence to date that the amount of thiomersal used in vaccines poses a health risk (World Health Organization, 2012). However, concerns are still raised in the scientific community regarding the safety of the use of ethylmercury in vaccines and the lack of precise regulations at EU level (Ruggieri, Majorani, Domanico, & Alimonti, 2017).

Mercury ranks 3rd and methylmercury 116th (out of a total of 275 substances) on the “ATSDR 2017 Substance Priority List” of the US Agency for Toxic Substances and Disease Registry (US Agency for Toxic Substances and Disease Registry (ATSDR), 2017).

According to the harmonised classification and labelling (ATP01) approved by the European Union, elemental mercury is a hazardous substance, which is fatal if inhaled (Acute Tox.2, “H330”), may damage the unborn child (Repr. 1B, “H360”), causes damage to organs through prolonged or repeated exposure (STOT RE 1, “H372” – Central Nervous System) and is very toxic to aquatic life (Aquatic Acute 1, “H400”) and with long-lasting effects (Aquatic Chronic 1, “H410”) (European Chemicals Agency, ECHA, n.d.).

Based on a systematic review of the literature, Grandjean and Landrigan suggested in 2006 that mercury and methylmercury are suspected neurotoxicants. The same authors updated their review of the existing data and noted that methylmercury is a developmental neurotoxicant, at much lower exposures than the concentrations that affect adult brain function. Genetic polymorphisms increase the vulnerability of the developing brain (Grandjean & Landrigan, Developmental neurotoxicity of industrial chemicals., 2006), (Grandjean & Landrigan, Neurobehavioural effects of developmental toxicity, 2014).

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According to the International Agency for Research on Cancer (IARC), methylmercury compounds are possibly carcinogenic to humans (Group 2B). Metallic mercury and inorganic mercury compounds are classified in Group 3 (not classifiable as to their carcinogenicity to humans) (International Agency for Research on Cancer, World Health Organization, 1993). The Commission for the Investigation of Health Hazards of Chemical Compounds of the Germany Research Foundation (DFG) placed organic and inorganic mercury compounds in Category 3B (substances that cause concern that they could be carcinogenic for man but cannot be assessed conclusively because of lack of data) (Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Germany Research Foundation, 2013). (Deutsche Forschungsgemeinschaft, 2020)

Mercury and mercury compounds are on the Proposition 65 list (Chemicals known to the State of California to Cause Cancer or Reproductive Toxicity) because they can cause birth defects or other reproductive harm. Methylmercury compounds are also on the Proposition 65 list because they can cause cancer (The Office of Environmental Health Hazard Assessment (OEHHA), State of California, USA).

According to the IRIS database, elemental mercury is not classifiable as to human carcinogenicity (Cat D) and methylmercury is a possible human carcinogen, for which human carcinogenicity data are inadequate (Cat C).

The Japanese GHS Classification classifies mercury as causing damage to organs through prolonged or repeated exposure (STOT RE 1 – nervous system, cardiovascular system, blood, liver, gingiva), as a reproductive toxicant (Category 1A), as Category 2 for mutagenicity, and does not classify it in terms of carcinogenicity.

15.1.1.2 Knowledge gaps

The toxic effects of methylmercury at the levels of exposure found in the general population due to fish consumption are not fully understood. New developments in epidemiological studies have indicated that n-3 long-chain polyunsaturated fatty acids in fish may counteract negative effects from methylmercury exposure that could impact the safety of the tolerable weekly intake (TWI) established by EFSA (Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment, 2018). The risk associated with dental amalgams is not fully understood (Bengtsson & Lars, 2017), (Bentung Lygre, Haug, Skjærven, & Björkman, 2016). Exposure to mercury has been linked with Alzheimer's disease, but further research is required (Mutter, Curth, Naumann, Deth, & Walach, 2010). Mercury has possible endocrine disruptive effects, which have raised public concern, but further investigation is required (Rana, 2014), (Iavicoli, Fontana, & Bergamaschi, 2009), (Rahman, Kumarathasan, & Gomes, 2016).

Additional prospective studies, which will include speciation analysis of the different forms of mercury, are needed for the investigation of the potential links of mercury to the metabolic syndrome, immunotoxicity and cardiovascular effects (Roy, Tremblay, & Ayotte, 2017), (Maqbool, Niaz, Ismail Hassan, Khan, & Abdollahi, 2017), (Gardner & Nyland, 2016), (Genchi, Sinicropi, Carocci, Lauria, & Catalano, 2017). A recent review and meta-analysis of Environmental toxic metal contaminants and risk of cardiovascular disease was published by Chowdhury et al. (2018) found that mercury was not associated with any cardiovascular outcomes (Chowdhury, et al., 2018). In an accompanying editorial, the difficulties of taking into account fish consumption in the analyses were reported and the authors suggested that the previous findings had to be taken with caution (Tellez-Plaza, Guallar, & Navas-Acien, 2018).

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15.1.2 Exposure characteristics

15.1.2.1 Environmental behaviour

Mercury is found in the environment in the metallic form and in various inorganic and organic complexes. The sources are both natural and anthropogenic.

The natural global bio-geochemical cycling of mercury is characterised by degassing of the element from soils and surface waters, atmospheric transport, deposition of mercury back to land and surface water, sorption onto soil or sediment particles and revolatilisation from land and surface water. This emission, deposition and revolatilisation creates difficulties in tracing the movement of mercury to its sources. Hg can be released into the air through weathering of rock containing Hg ore, or through human activities, such as incineration and burning of fossil fuels. The life-time of mercury in the atmosphere varies between 0.8 – 2 years (Gworek, Bemowska-Kałabun, Kijeń, & Wrzosek-Jakubowska, 2016). Hg released in atmosphere is a significant indirect risk to human health, since it is the main way in which it travels around the globe and gets deposited in water bodies and on land. For this reason, mercury is global pollutant. Once in the environment, interconversion between the different forms of Hg can occur. Particulate-bound Hg can be converted to insoluble Hg sulfide and can be precipitated or bioconverted into more volatile or soluble forms that re-enter the atmosphere or are bioaccumulated in aquatic and terrestrial food chains (National Research Council, Committee on the Toxicological Effects of Methylmercury, Board on Environmental Studies and Toxicology, 2000).

Water contamination can occur from run-off water, contaminated by either natural or anthropogenic sources or from air deposition. The biggest risk to human health is mercury in aquatic environments, because it stays there for a very long time (the lifetime of mercury in the upper oceans is 20 - 30 years and can be hundreds of years in the deep ocean) and it gets converted by microorganisms to the very toxic organic form, methylmercury (Gworek, Bemowska-Kałabun, Kijeń, & Wrzosek-Jakubowska, 2016).

Methylmercury bioaccumulates inside biological organisms, since its excretion is slower than its uptake and biomagnifies as predatory animals consume prey that already accumulated mercury (Hanna, Solomon, Poste, Buck, & Chapman, 2015), (Lavoie, Jardine, Chumchal, Kidd, & Campbell, 2013). The concentration of mercury in fish species is influenced by the position of the species in the food web (e.g. it is higher in predators, such as swordfish and lower in low-end species, such as sardines), but also on the region. In Europe, the highest concentrations are found in the Mediterranean Sea (Miklavčič Višnjevec, Kocman, & Horvat, Human mercury exposure and effects in Europe), which may be due to favourable conditions for the generation of methylmercury (European Environment Agency , 2018), (Cossa & Coquery, 2005).

Mercury deposited on land also enters the food-chain, as for example, in the case of rice grown on contaminated soil (Rothenberg, Windham-Myers, & Creswell, 2014). Because rice is grown in water, methylmercury may be formed and absorbed in the grain (Tanner, et al.).

15.1.2.2 Human exposure

Humans face exposure risks to all forms of mercury from numerous sources and routes of exposure. Human exposure to mercury may occur through the following routes:

- ▶ **Dermal:** Mercury is a suspected skin sensitiser and allergen, but it is not significantly absorbed through the skin and so this is not a significant route.
- ▶ **Inhalation:** Inhalation of mercury vapours may occur in industrial processes, but for the general population, this route is not a significant route.

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- ▶ **Oral:** This is a significant route of exposure for the general population. Human exposure occurs mainly through the consumption of contaminated fish and shellfish, with methylmercury presenting the most significant risk. Elemental mercury from ingestion is poorly absorbed with a bioavailability of less than 0.01% (Park & Zheng, 2012).
- ▶ **Trans-placenta:** This is a significant route of human exposure, since mercury crosses the placenta and results in foetal exposure.

Sources and routes of exposure vary geographically in a significant way and this complicates the development of strategies able to protect populations in specific locations. In developing nations, exposure may result from occupational activities such as artisanal and small-scale mining, religious and cultural practices and diet based almost exclusively on fish consumption. The most significant source of human exposure to mercury in Europe is through the diet. Populations consuming a lot of fish, such as in coastal regions - the Mediterranean region of Europe or Arctic regions - are the most vulnerable. Exposure levels are influenced also by the type of fish consumed (eating predatory fish entails a higher risk). On the other hand, fish consumption provides omega-3 fatty acids, which have protective health effects. To balance the health benefits provided by seafood consumption with the negative effects from possible exposure to mercury, the European and many National Food Safety Authorities developed dietary advice. A recent study by Kirk et al. in Denmark, showed that providing dietary advice to pregnant women to consume preferably non-predatory fish, was effective in lowering their exposure to methylmercury as determined by mercury analysis in hair (Kirk, Jørgensen, Nielsen, & Grandjean, 2017), (European Environment Agency , 2018). Rise-based diets are an increasing risk factor (World Health Organization (WHO), 2010).

Mercury exposure from non-dietary sources is small for the general population. Inhaled mercury from ambient air is very efficiently absorbed, but for the general population this is not a significant risk since the levels of mercury in outdoor air are usually very low. Mercury amalgam used in dental fillings and broken mercury-containing products (e.g. thermometers) may also lead to minor exposures. Exposure to thimerosal, an ethylmercury-sulfidobenzoate used as preservative in several human vaccines, is now very limited in Europe. The European Centre for Disease Prevention and Control and the World Health Organization concluded that thimerosal is not harmful, based on assessment of the current scientific evidence (European Environment Agency , 2018).

Local communities living near mercury-polluted sites, such as the Almaden mining area in Spain, may face increased risk of becoming exposed. One example is the former mining town of Idrija, Slovenia, where locally produced foodstuffs (fish, mushrooms, chicory) have been found to contain increased mercury concentrations (Miklavčič, Mazej, Jačimović, Dizdarevič, & Horvat, 2013).

Human exposure to mercury begins at the time of conception and continues beyond the critical time of gestation throughout infancy, childhood and into adulthood. Prenatal exposures of the foetus relate to the sources of the mother's exposure, with the diet being very important. Another source of exposure may be Hg vapours released from dental amalgams, which contain up to 50% elemental mercury (Bentung Lygre , Haug , Skjærven , & Björkman, 2016).

During pregnancy, maternal exposure to mercury can damage the neurodevelopment of the foetus, with noticeable effects on behaviour, cognition, motor skills and the immune and reproduction systems later in life (Rice & Barone Jr., 2000). Infants are at higher risk than older children and adults. This may relate to their highly efficient gastrointestinal absorption, physiological immaturity of homeostasis and detoxification mechanisms. The most significant pathway of infant exposure is breast milk consumption, but use of specific mercury-containing products, such as teething powders, soaps, may contribute (World Health Organization, 2010). Both organic and inorganic

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mercury occur in breast milk, but the physiology of the mammary gland causes preferential enrichment of inorganic mercury. Inorganic mercury rapidly enters the plasma and therefore, the breast milk. Methylmercury partitions preferentially to erythrocytes (Ask Björnberg, Vahter, & Petersson-Grawé, 2003).

Inorganic mercury salts are not lipid soluble; hence, they do not readily cross the blood-brain barrier or blood-placenta barrier (Dart & Sullivan, 2004). Inhalation is a major exposure route of elemental mercury in the form of mercury vapor. Inhaled mercury vapor is readily absorbed, at a rate of approximately 80%, in the lungs, and quickly diffused into the blood and distributed into all of the organs of the body. Elemental mercury can cross the blood-brain barrier and blood-placenta barrier as well as the lipid bilayers of cellular and intracellular organellar membranes (Park & Zheng, 2012). Elemental mercury is poorly absorbed in the gastrointestinal tract (less than 0.01%) (Von Burg, 1995).

15.1.2.3 Human Biomonitoring (HBM)

Recently, Basu et al. (2018) reviewed the state-of-the-science of mercury biomarkers in human populations worldwide between 2000-2018. A systematic search of the peer-reviewed literature resulted in collection of 424858 mercury biomarker measurements from 335991 individuals represented in 312 scientific articles from 75 countries. This assessment showed that general background populations with insignificant exposures have mercury levels that generally fall under 5 µg/L in blood, 2 µg/g in hair and 3 µg/L in urine. Four populations of concern were identified: a) Arctic populations, who consume fish and marine mammals; b) tropical riverine communities (especially Amazonian) who consume fish and, in some cases, may be exposed to mining; c) coastal and/or small-island communities who substantially depend on seafood; and d) individuals who either work or reside among artisanal and small-scale gold mining sites. The authors concluded that all populations worldwide are exposed to some amount of mercury and that there is great variability in exposures within and across countries and regions. Only limited data exist for many geographic regions and subpopulations, which hinders evidence-based decision making. This information-gap must be addressed, since it is critical in helping understand exposures, both at EU-level and globally, particularly in light of certain stipulations in the Minamata Convention on Mercury (Basu, et al., 2018).

Miklavčič Višnjevec et al. (2014) reviewed published studies from 2000 to 2014 on European populations. The exposure and effects studies were compared with known Hg levels in environmental compartments by mapping the various population groups studied and taking into account known sources of Hg. The spatial distribution trends confirmed that the highest exposure levels to Hg, mostly as methylmercury (MeHg), are found in coastal populations, which consume more fish than inland populations. Fewer studies addressed exposure to elemental Hg through inhalation of Hg in air and inorganic Hg in food, particularly in highly contaminated areas. Overall, at the currently low exposure levels of Hg prevalently found in Europe, further studies are needed to confirm the risk to European populations, taking into consideration exposure to various Hg compounds and mixtures of stressors with similar end-points, nutritional status, and a detailed understanding of Hg in fish present in European markets (Miklavčič Višnjevec, Kocman, & Horvat, Human mercury exposure and effects in Europe, 2014).

The United States (U.S.) Centers of Disease Control and Prevention follows the exposure of the general U.S. population to mercury. Updated data on blood mercury species (inorganic, ethyl and methyl mercury) were published in 2019 (U.S. Centers for Disease Control and Prevention, 2019).

DEMOCOPHES, carried out in 2010-2012, was the first Europe-wide harmonised Human Biomonitoring study. It investigated the mercury exposure of children ages 6-11 and their mothers in 17 countries (BE, CH, CY, CZ, DE, DK, ES, HU, IE, LU, PL, PT, RO, SI, SK, SE, UK), using

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scalp-hair samples. The mercury concentrations found at European level, were Mean=0.14 µg/g, P₉₀=0.82 µg/g for children and Mean=0.22 µg/g, P₉₀=1.3 µg/g for mothers. The guidance value used for evaluation of the results, was the JECFA recommended Tolerable Daily Intake (TDI) = 2.3 µg/g (Joint FAO/WHO Expert Committee on Food Additives (JECFA), 2006). The results showed that Spain and Portugal had the highest exposures (7 and 5 times above the European mean), which was attributed to sea-food consumption (Den Hond, et al., 2015). Exposure was significantly lower (2 times above the European mean) in Cyprus, an island state with high sea-food consumption, which may be due to the consumption of primarily smaller non-piscivorous fish. Based on the DEMOCOPHES results, it was estimated that nearly 1.9 million babies are born yearly in Europe with mercury levels above the recommended safe limit, with an estimated associated economic cost of at least EUR 9 billion (Bellanger, et al., 2013). In DEMOCOPHES, the sampling was not representative of the national populations. Further investigations are needed, using representative data, to assess the body burden of Europeans and the sources of exposure. It is also important to follow time trends, which will contribute to the effectiveness assessment of European policy actions and of the Minamata Convention.

A summary of available Human Biomonitoring from EU countries on mercury exposure are summarised in a report from the World Health Organization (2015) (World Health Organization (WHO), 2015) and are shown in the table below. Additional data are provided by Ruggieri et al. (2017) (Ruggieri, Majorani, Domanico, & Alimonti, 2017), who described current HBM studies on Hg exposure in children. Additional results were from further review of the scientific literature or provided by internal HBM4EU partners. In 2018, Steckling et al. reviewed available biomarkers of exposure, exposure determinants, reference and exposure limit values for mercury and other environmental stressors, in a review developed in the frame of the HEALS project (Steckling, et al., 2018). In 2019, the results on men and lactating women from the first Slovenian HBM study were reported (Snoj Tratnik, et al., 2019).

Table 15-1: Summary of European HBM studies on mercury exposure

Country	Study	Population (N)	Total mercury				Methyl-mercury	Ref.
			Blood (ng/mL unless otherwise stated)	Urine		Hair (µg/g)	Hair (µg/g)	
				(µg/g creat)	(µg/L)			
Austria	2008-2010	Children Age: 6-11 y N=?? (A total of 104 samples from adults/children were analysed for MetHg. 50 children participated, but it is not specified if in how many hair samples were assessed for MetHg)	-	-	-	-	Median=0.006	(Hohenblum, et al., 2012)
		Adults (parents) Age: 25-50y N=?? (A total of 104 samples from adults/children were analysed for MetHg. 100 children participated, but it is not specified if in how many hair MetHg was assessed).	-	-	-	-	Median=0.064	
Belgium (Flanders)	FLESH (2007-2011)	Mothers Age: 20-40 y N = 242	-	-	-	GM=0.35 P ₉₀ =0.82	GM=0.26 P ₉₀ =0.65	(Schoeters, et al., 2012)
		Adolescents Age: 14-15 y N = 206	-	-	-	GM=0.19 P ₉₀ =0.47	GM=0.12 P ₉₀ =0.35	

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Country	Study	Population (N)	Total mercury				Methyl-mercury	Ref.
			Blood (ng/mL unless otherwise stated)	Urine		Hair (µg/g)	Hair (µg/g)	
				(µg/g creat)	(µg/L)			
	DEMOCOPHES-BE (2010-2012)	Children Age: 6-11y N=127	-	-	-	GM=0.204 (0.172,0.241)	-	(Den Hond, et al., 2015)
		Mothers Age <45y N=127	-	-	-	GM=0.368 (0.313,0.431)	-	
Croatia	PhD Thesis HBM survey in Croatian capital city 2008-2009	Mothers Age=25 to 35 (vegetarian and nonvegetarian) N=102	0.120-13.32 µg/l (Median= 1.840)	Creatinine concentrations 0,509 to 2,601 g/l (Median 1.176)	Concentrations of Hg in urine adjusted to creatinine 0.089 to 5.743 µg/g (Median= 0.689)	0.027-3.899 µg/g (Median= 0.418)		(Janev Holcer, 2010)
Cyprus	DEMOCOPHES-CY (2010-2012)	Children Age: 6-11y N=60	-	-	-	GM=0.326 (0.257,0.413)	-	(Den Hond, et al., 2015)
		Mothers Age <45y N=60	-	-	-	GM=0.462 (0.369,0.578)	-	
Czech Republic	CZ-HBM (2001-2003)	Children Age: 8-10y	GM=0.43 P95=1.44 N=333	GM=0.45 P95=4.18 N=619	-	-	-	(Batáriová, et al., 2006), (Černá, Krsková, Čejchanová, & Spěváčková, 2012)
		Adults Age: 18-58 y N=1188	GM=0.82 P95=3.45	GM=0.61 P95=6.8	-	-	-	
	CZ-HBM 2008	Children Age: 8-10y	GM=0.45 P95=1.39 N=382	-	GM=0.26 P95=2.19 N=364	GM=0.18 P95=0.61 N=316	-	

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Country	Study	Population (N)	Total mercury			Methyl-mercury	Ref.
			Blood (ng/mL unless otherwise stated)	Urine		Hair (µg/g)	
				(µg/g creat)	(µg/L)		
	CZ-HBM (2005-2009)	Adults Age: 18-58 y N=1227	GM=0.6 P95=0.75	-	-	-	
	DEMOCOPHES-CZ (2010-2012)	Children Age: 6-11y N=120	-	-	-	GM=0.098 (0.083, 0.116)	(Den Hond, et al., 2015)
		Mothers Age <45y N=120	-	-	-	GM=0.156 (0.133,0.183)	
Denmark	DEMOCOPHES-DK (2010-2012)	Children Age: 6-11y N=144	-	-	-	GM= 0.250 (0.211,0.295)	(Den Hond, et al., 2015)
		Mothers Age <45y N=144	-	-	-	GM= 0.391 (0.333,0.458)	
France	ENNS (2006-2007)	Adults 18–74 y N=365	-	-	GM=0.59 P95=1.90	-	(Fréry, Vandentorren, Etcheverris, & Fillol, 2012)
		Children Age: 3–17 y N=1364	-	-	GM=0.37 P95=1.20	-	

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Country	Study	Population (N)	Total mercury				Methyl-mercury	Ref.
			Blood (ng/mL unless otherwise stated)	Urine		Hair (µg/g)	Hair (µg/g)	
				(µg/g creat)	(µg/L)			
Germany	Environmental Specimen Bank	Adults Age: 20-29 N= 480/ year from 4 sampling locations	Data available for time trends 1995-2017	Data available for time trends 1995-2017	Data available for time trends 1995-2017			www.umweltprobenbank.de
	GerES I (1985-86)	Adults Age: 25-69ar N=2519	-	-	-	-	-	(Kolossa-Gehring, et al., 2012), (Schulz, Wilhelm, Heudorf, & Kolossa-Gehring, Reprint of "Update of the reference and HBM values derived by the German Human Biomonitoring Commission", 2012)
	GerES II (1990-92)	Adults Age: 18-79 y N=4287	GM=0.5 P ₉₅ =2.0	-	GM=0.53 P ₉₅ =3.7	-	-	
		Children Age: 6-17 y N=812	GM=0.33 P ₉₅ =1.4	-	GM=0.54 P ₉₅ =3.9	-	-	
	GerES III (1998)	Adults Age: 18-69 y N=4822	GM=0.61 P ₉₅ =2.4	-	GM=0.4 P ₉₅ =3.0	-	-	
	GerES IV (2003-2006)	Children Age: 3-14 y N=1552	GM=0.23 P ₉₀ =0.3	-	GM<0.1 P ₉₀ =0.3	-	-	

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Country	Study	Population (N)	Total mercury				Methyl-mercury	Ref.
			Blood (ng/mL unless otherwise stated)	Urine		Hair (µg/g)	Hair (µg/g)	
				(µg/g creat)	(µg/L)			
	GerES V	Children and adolescents Age: 3- 17						Paper in preparation
	DEMOCOPHES-DE (2010-2012)	Children Age: 6-11y N=120	-	-	-	GM=0.055 (0.046, 0.065)	-	(Den Hond, et al., 2015)
		Mothers Age <45y N=120	-	-	-	GM=0.107 (0.092,0.126)	-	
Hungary	DEMOCOPHES-HU	Children Age: 6-11y N=119	-	-	-	GM=0.025 (0.021,0.029)	-	(Den Hond, et al., 2015)
		Mothers Age <45y N=119	-	-	-	GM=0.039 (0.033,0.045)	-	
Ireland	DEMOCOPHES-IE	Children Age: 6-11y N=120	-	-	-	GM=0.097 (0.082,0.114)	-	(Den Hond, et al., 2015)
		Mothers Age <45y N=120	-	-	-	GM=0.162 (0.139,0.190)	-	
Italy	PROBE (2008-2010)	Adolescents Age: 13–15 y N=252	GM=0.84 P95=3.55	-	-	-	-	(Pino, Amato, Alimonti, Mattei, & Bocca, 2012)

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Country	Study	Population (N)	Total mercury			Methyl-mercury	Ref.	
			Blood (ng/mL unless otherwise stated)	Urine		Hair (µg/g)		
				(µg/g creat)	(µg/L)			Hair (µg/g)
	2007-2009	Pregnant women	Median=2.35 ng/g P ₇₅ =3.98 ng/g N=606	-	-	Median=0.78 P ₇₅ =1.28 N=604	Median=1.38 P ₇₅ =1.85 N=220	(Valent, et al., 2013)
	PHIME Project Site A: North Italy - NACII	Children Age: 7y N=200	-	-	-	Median=0.596 P ₇₅ = 0.996 N=200	-	(Pino, et al., 2018)
	PHIME Project Site B: South Italy	Children Age: 6-11 y N=299	-	-	-	Median=0.477 P ₇₅ =0.747 N=299	-	
Luxembourg	DEMOCOPHES -LU	Children Age: 6-11y N=56	-	-	-	GM=0.181 (0.142,0.229)	-	(Den Hond, et al., 2015)
		Mothers Age <45y N=56	-	-	-	GM=0.387 (0.308,0.485)	-	
Poland	DEMOCOPHES-PL	Children Age: 6-11y N=120	-	-	-	GM=0.070 (0.060,0.083)	-	(Den Hond, et al., 2015)
		Mothers Age <45y N=120	-	-	-	GM=0.135 (0.116,0.159)	-	
Portugal	DEMOCOPHES-PT	Children Age: 6-11y N=120	-	-	-	GM=1.033 (0.873,1.222)	-	(Den Hond, et al., 2015)

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Country	Study	Population (N)	Total mercury				Methyl-mercury	Ref.
			Blood (ng/mL unless otherwise stated)	Urine		Hair (µg/g)	Hair (µg/g)	
				(µg/g creat)	(µg/L)			
		Mothers Age <45y N=120	-	-	-	GM=1.200 (1.023,1.406)	-	
Romania	DEMOCOPHES-RO	Children Age: 6-11y N=120	-	-	-	GM=0.085 (0.072,0.101)	-	(Den Hond, et al., 2015)
		Mothers Age <45y N=120	-	-	-	GM=0.100 (0.085,0.117)	-	
Slovakia	DEMOCOPHES-SK (2010-2012)	Children Age: 6-11y N=129	-	-	-	GM= 0.092 (0.078,0.109)	-	(Den Hond, et al., 2015)
		Mothers Age <45y N=129	-	-	-	GM=0.132 (0.112,0.154)	-	
Slovenia	National HBM Survey (2007-2009)	Adults Age: 20-40 y N=274	GM=1.07 P95=4.03	GM=0.50 P95=3.44	-	GM=0.23 P95=0.89	-	(Snoj Tratnik J, 2012)
	DEMOCOPHES-SI (2010-2012)	Children Age: 6-11y N=120	-	-	-	GM= 0.169 (0.142,0.200)	-	(Den Hond, et al., 2015)
		Mothers Age <45y N=120	-	-	-	GM= 0.255 (0.217,0.299)	-	

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Country	Study	Population (N)	Total mercury				Methyl-mercury	Ref.
			Blood (ng/mL unless otherwise stated)	Urine		Hair (µg/g)	Hair (µg/g)	
				(µg/g creat)	(µg/L)			
Spain	BIOVAL programme (2016)	Children Age: 6-11y N=611 (Valencia Region)	-	-	-	GM= 0.79 (Range 0.03-8.71) P75=1.57 P95=3.25	-	(Pérez, et al., 2019)
	ISCIII pilot study (2009 - 2010)	Adults Age: 23–66 y N=170	-	GM=1.23 P ₉₀ =2.72 P ₉₅ =3.30	-	-	-	(Castaño, et al., 2012)
	Yusà et. al. (2017)	Breastfeeding mothers Age: 20-45y N=120				GM=1.22 (Range= 0.07 to 6.87)		(Yusà, et al., 2017)
	Roca et. al (2016)	Children Age: 6-11y N=120 (Valencia)		GM= 0.730 P ₉₅ =2.64 Max= 6.21				(Roca, Sánchez, Pérez, Pardo, & Yusà, 2016)
	Batista et. al. (1996)	Children Age: 6-16y N= 233				GM=0.77		(Batista, Schuhmacher, Domingo, & Corbell, 1996)
	DEMOCOPHES-ES (2010-2012)	Children Age: 6-11y N=120	-	-	-	GM=0.884 (0.747,1.046)	-	(Den Hond, et al., 2015)

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Country	Study	Population (N)	Total mercury				Methyl-mercury	Ref.
			Blood (ng/mL unless otherwise stated)	Urine		Hair (µg/g)	Hair (µg/g)	
				(µg/g creat)	(µg/L)			
		Mothers Age <45y N=120	-	-	-	GM=1.486 (1.267,1.744)	-	
Sweden	DEMOCOPHES-SE (2010-2012)	Children Age: 6-11y N=100	-	-	-	GM= 0.181 (0.153,0.214)□	-	(Den Hond, et al., 2015)
		Mothers Age <45y N=100	-	-	-	GM=0.252 (0.215,0.295)	-	
Switzerland	DEMOCOPHES-CH (2010-2012)	Children Age: 6-11y N=120	-	-	-	GM= 0.076 (0.065,0.090)	-	(Den Hond, et al., 2015)
		Mothers Age <45y N=120	-	-	-	GM= 0.153 (0.131,0.180)	-	
United Kingdom	DEMOCOPHES-UK (2010-2012)	Children Age: 6-11y N=21	-	-	-	GM=0.192 (0.163,0.228)	-	(Den Hond, et al., 2015)
		Mothers Age <45y N=21	-	-	-	GM=0.153 (0.130,0.180)	-	
Italy, Greece, Slovenia, and Croatia	PHIME project*	Mothers Age= 32 (median) N=1282	Median= 2.4 ng/g, P80=4.4 ng/g N=733	-	-	Median=0.70 P ₈₀ =1.46 N=1282	-	(Barbone , et al.)

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Country	Study	Population (N)	Total mercury				Methyl-mercury	Ref.
			Blood (ng/mL unless otherwise stated)	Urine		Hair (µg/g)	Hair (µg/g)	
				(µg/g creat)	(µg/L)			
17 EU countries	DEMO-COPHES (2010-2012)	Children Age: 6-11y N=1844	-	-	-	GM=0.15 P ₉₅ =0.80	-	(Den Hond, et al., 2015)
		Mothers Age <45y N=1844	-	-	-	GM=0.23 P ₉₅ =1.20	-	

*PHIME project results of total mercury in other matrices:
Breast milk N=819, Median 0.2 ng/g, P80 0.4 ng/g;
Cord blood N=1078, Median 3.6 ng/g, P80 7.8 ng/g

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HBM is generally a cross-sectional study (one time or over a short period sampling). Some national HBM cross-sectional surveys were complemented with longitudinal birth cohort studies that allowed to assess perinatal exposure (by biomarkers measured in specimens of the pregnant mother, in cord blood, or in breast milk) and, following up the children, to: (i) describe the degree of individual perinatal Hg exposure and the internal dose during pregnancy; (ii) monitor temporal and spatial patterns of exposure from birth; (iii) evaluate the health effects occurring on foetal and infant growth, and during childhood development; and (iv) link environmental factors and exposures to health, with the aim of informing and orienting public policy decision-making (Ruggieri, Majorani, Domanico, & Alimonti, 2017). The following table, reproduced from Ruggieri et al. (2017) (Ruggieri, Majorani, Domanico, & Alimonti, 2017), provides an overview of the European birth cohort studies, which included investigation of mercury (collected from the webpage www.birthcohorts.net) and some smaller scale longitudinal research.

Table 15-2: Overview of European birth national cohorts, which included investigation of mercury

Country	Birth Cohort	Metals	Enrollment Period	No. of Children at Birth	Ref.
Faroe Islands	Faroese: Children's Health and the Environment in the Faroes	Hg, Pb, Se	1986–2009	2351	(Grandjean, et al., 1997), (Grandjean, Murata, Budtz-Jørgensen, & Weihe, 2004)
United Kingdom	ALSPAC—The Avon Longitudinal Study of Parents and Children	As, Cd, Hg, Mn, Pb, Se	1991–1992	14,062	(Golding, et al., 2013)
Denmark	DNBC—Danish National Birth Cohort	Hg	1996–2002	96986	(Olsén, et al., 2001)
Spain	INMA—Environment and Childhood	Hg, Pb, TMS	1997–2008	3757	(Ramón, et al., 2011)
Norway	MoBa—Norwegian Mother and Child Cohort Study	Hg	1999–2008	100000	(Vejrup, et al., 2014)
Germany	Duisburg cohort	Cd, Hg, Pb, Se	2000–2003	234	(Wilhelm, et al., 2008)
Poland	Kraków cohort	Cd, Hg, Pb	2000–2003	505	(Jedrychowski, et al., 2007)
	REPRO_PL—Polish Mother and Child Cohort	Cd, Hg, Pb, Se, Zn, Cu	2007–2011	1800	(Polanska, et al., 2011)
Slovakia	PCB cohort—Early Childhood Development and PCB exposures in Slovakia	Hg, Pb	2001–2003	1134	(Sonneborn, et al., 2008)
Finland	LUKAS cohort: Finnish cohort	As, Cd, Hg, Pb, Se	2002–2005	442	(Leino, et al., 2013)
France	PÉLAGIE—Endocrine disruptors: longitudinal study on pregnancy abnormalities, infertility, and childhood	Hg	2002–2006	3421	(Guldner, Monfort, Rouget, Garlantezec, & Cordier, 2007)
	ELFE: French longitudinal study of children	Al, As, Cd, Hg, Pb	2011–2012	20000	(Vandentorren, et al., 2009)

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Country	Birth Cohort	Metals	Enrollment Period	No. of Children at Birth	Ref.
Italy	Trieste Cohort: Trieste child development cohort	Hg, Pb, Se, Zn	2007–2009	900	(Valent, et al., 2013)
Greece	RHEA—Mother Child Cohort in Crete	As, Cd, Hg, Mn, Pb	2007–2008	1500	(Vardavas, et al., 2009)
Croatia	Implementation of Human Biomonitoring survey of prenatal exposure to mercury in two Croatian regions using the standardised WHO methodology – Mother Child study in Croatia	Hg	2015 - 2016	290	(Capak, et al., 2016)
Italy, Greece, Slovenia, and Croatia	NACII—Mediterranean cohort study, (within PHIME project)	Cd, Hg, Pb, Mn, Se, Zn	2006–2011	1700	(Valent, et al., 2013)

INMA: *Infancia y Medio Ambiente* (Spanish: *Environment and Childhood*); REPRO_PL: *Polish Mother and Child Cohort*; PCB: *polychlorinated biphenyl*; PÉLAGIE: *Perturbateurs Endocriniens: Étude Longitudinale sur les Anomalies de la Grossesse, l'Infertilité et l'Enfance* (French: *Endocrine Disruptors: Longitudinal Study on Disorders of Pregnancy, Infertility and Children*); ELFE: *Etude Longitudinale Française depuis l'Enfance* (French *Longitudinal Study of Children*); NACII: *Northern Adriatic Cohort*; PHIME: *Public Health Impact of long-term low-level Mixed Element Exposure*.

An overview of reference values for mercury in blood and urine are provided in Saravanabhavan et al. (2017) (Saravanabhavan, et al., 2017) and summarised in the tables below.

Table 15-3: Overview of reference values for mercury (total) in blood

Mercury (total)						
Country: Survey	Study period	Population (years)	N	Exclusion criteria	RV ₉₅ (µg/L)	Ref.
Brazil	2006	18–65	593		4	(Kuno, Roquetti, Becker, Seiwert, & Gouveia, 2013)
Czech Republic: HBM project	2005–2009	8–10	723		1.4	(Černá, Krsková, Čejchanová, & Spěváčková, 2012)
Czech Republic: HBM project	2005–2009	18–58	1221	A	2.6	(Černá, Krsková, Čejchanová, & Spěváčková, 2012)
Germany: GerES IV	2003–2006	3–14	891	B	0.8	(Schulz, Wilhelm, Heudorf, & Kolossa-Gehring, Reprint of "Update of the reference and HBM values derived by the German Human Biomonitoring Commission", 2012)
Germany: GerES III	1997–1999	18–69	2310	B	2.0	(Wilhelm, Ewers, & Schulz, 2004)
Italy: PROBE	2008–2010	18–65	1423		5.16	(Alimonti, Bocca, Mattei, & Pino, 2011)
Korea: KorSEP III	2008	≥20	1963	C	9.42	(Lee, et al., 2012)

A: average fish consumption of ≥1 time per week

B: average fish consumption of >3 times per month

C: fish consumption within 72 h of sample collection

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Table 15-4: Overview of reference values for mercury (inorganic) in urine

Mercury (inorganic)						
Country: Survey	Study period	Population (years)	N	Exclusion criteria	RV ₉₅ (µg/L)	Ref.
Czech Republic: HBM project	2005–2009	8–10	723		3	(Černá, Krsková , Čejchanová , & Spěváčková, 2012)
Czech Republic: HBM project	2005–2009	18–58	1227		9	(Černá, Krsková , Čejchanová , & Spěváčková, 2012)
Germany: GerES IV	2003–2006	3–14	1612	A, C	0.4	(Schulz, Wilhelm, Heudorf, & Kolossa-Gehring, Reprint of "Update of the reference and HBM values derived by the German Human Biomonitoring Commission", 2012)
Germany: GerES III	1997–1999	18–69	1560	A, C	1.0	(Wilhelm, Ewers, & Schulz, 2004)
Belgium	2010–2011	>18	1001	B	1.88	(Hoet, Jacquerye, Deumer, Lison, & Haufroid, 2013)

A: creatinine levels <0.3 or >3.0 g/L, B: occupational exposure, C: presence of dental amalgam fillings

15.1.2.4 Health based guidance values available for HBM data

15.1.2.4.1.1 General population

The following table summarises the available public health risk-based values in terms of biomarker concentrations and has been adopted from Ruggieri et al. (2017) (Ruggieri, Majorani, Domanico, & Alimonti, 2017).

Table 15-5: Summary of available public health risk-based values for mercury

	Reference population	HBM-I	HBM-II	NCR	JECFA	Bellanger et al. (2013) (Bellanger , et al., 2013)
Mercury (total) in urine	Children, women of child-bearing age / adults	7 µg/L (5 µg/g creat.)	25 µg/L (20 µg/g creat.)			
Mercury (total) in blood	Children, women of child-bearing age / adults	5 µg/L	15 µg/L			
Mercury in hair (dry weight)	Children, women of child-bearing age			1 µg/g	2.3 µg/g	0.58 µg/g
Mercury (total) in cord blood	-			5.8 µg/L		
Mercury (total) in maternal blood	Pregnant women			3.5 µg/L		

HBM: Human Biomonitoring

NCR: National Research Council

JECFA: Joint FAO/WHO Expert Committee on Food Additives

FAO: Food and Agriculture Organization of the United Nations
creat.: creatinine

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The German HBM Commission defined HBM-I and HBM-II values for total mercury in urine (HBM-I: 7 µg/L (5 µg/g creat.); HBM-II: 25 µg/L (20 µg/g creat.) and for total mercury in blood (HBM-I: 5 µg/L; HBM-II: 15 µg/L). The HBM-I value corresponds to the concentration of a substance in a human biological matrix below which no adverse health effects are expected.

The HBM-II value corresponds to the concentration above, which there is an increased risk of adverse health effects and is therefore an intervention or action threshold level.

No HBM values were set for hair by German HBM Commission (Schulz , Wilhelm , Heudorf, & Kolossa-Gehring, Update of the reference and HBM values derived by the German Human Biomonitoring Commission, 2011). The values derived for women of reproductive age are recommended for other groups of adults.

A guidance value for Hg in hair (2.3 µg/g dry weight) was defined by the Joint Food and Agriculture Organization of the United Nations and WHO (FAO/WHO) Expert Committee on Food Additives (JECFA), in order to protect foetus from neurotoxic effects. It is based on the provisional tolerable weekly intake (PTWI) limit of 1.6 µg/kg bw/week for MeHg and takes into in consideration the potential benefit of nutrients in fish (i.e., omega-3 fatty acids) against the MeHg toxicity (Joint FAO/WHO Expert Committee on Food Additives (JECFA), 2006).

The US EPA set a stricter RfD for chronic oral exposure to MeHg of 0.1 µg/kg bw/day for developmental neuropsychological impairment, which corresponds to 1 µg/g total Hg in hair for children and women in reproductive age (National Research Council, Committee on the Toxicological Effects of Methylmercury, Board on Environmental Studies and Toxicology, 2000), (Ruggieri, Majorani, Domanico, & Alimonti, 2017), but more recent calculations with data on developmental neurotoxicity at background exposure levels, resulted in the much lower biological limit in hair of 0.58 µg/g (Ruggieri, Majorani, Domanico, & Alimonti, 2017). Using the RfD value and assuming a ratio of MeHg in infant cord blood to maternal blood 1.7 : 1.1 (e.g., 70% higher in cord than maternal blood), a maternal total Hg blood safe-concentration was set at 3.5 µg/L and in cord blood at 5.8 µg/L (National Research Council, Committee on the Toxicological Effects of Methylmercury, Board on Environmental Studies and Toxicology, 2000), (Ruggieri, Majorani, Domanico, & Alimonti, 2017).

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15.1.2.5 Occupational population

The following recommendations are available:

Table 15-6: Recommended Biological Limit Values (BLV) for occupational exposure

Organisation	Biological Limit Value (BLV)	Ref.
The Scientific Committee on Occupational Exposure Limits (SCOEL), European Commission, Employment, Social Affairs & Inclusion	Blood: 10 µg Hg/l Urine: 30 µg Hg/g creatinine	(The Scientific Committee on Occupational Exposure Limits (SCOEL), 2007)
Finland/FIOH	BAL Metallic mercury and inorganic mercury: Urine: 140 nmol/L (28 µg/L) BAL inorganic mercury: Blood: 50 nmol/L (10 µg/L)	(INRS) (INRS)
Germany/ Deutsche Forschungsgemeinschaft (DFG)	BAT Value Mercury and inorganic compounds: Urine: 30 µg/L or 25 µg/g creat.	(INRS), (Schaller, 2003), (Deutsche Forschungsgemeinschaft (DFG), Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, 2017), (Deutsche Forschungsgemeinschaft, 2020)
France/INRS	Organic mercury: Maintain blood methylmercury (for occupational exposure) below 100 µg/L	(INRS)
UK/HSL	Biological Biomonitoring Guidance Value (BMGV) for inorganic mercury Urine: 20 µmol /mol creat. (conversion: 1µmol/mol = 1.17µg/g)	(HSL)
USA/ ACGIH	BEI Inorganic mercury: Urine: 20 µg/g creat.	(INRS)
Spain	VLB: Elemental mercury and inorganic compounds (2013) Total inorganic mercury in urine: 30 µg/ g creatinine Total inorganic mercury in blood: 10 µg/l	(Instituto Nacional de Seguridad y Salud en el Trabajo, 2018)

15.1.3 Policy relevance

15.1.3.1 European Policies

The European Commission adopted in 2005 the Community Strategy Concerning Mercury (European Commission, 2005), which includes a comprehensive plan to address mercury use and pollution and has resulted in the enhancement of Union law on mercury, including restrictions on the inclusion of mercury or mercury compounds in products, ban of exports of mercury from the EU and inclusion of provisions on mercury emissions in EU legislation to protect people against exposure. European legislations concerning mercury are described below.

15.1.3.2 Food safety

Limits on the mercury content of fish for human consumption for protecting human health are defined in *European Regulation (EC) No 1881/2006* (European Commission, 2006) and amended on Regulation No 629/2008 (European Commission, 2008). The maximum safe limit for most fish

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species for human consumption is currently 0.5 mg/kg wet body weight and for some predatory species such as swordfish and tuna, it is 1 mg/kg wet body weight. *Directive 2002/32/EC* sets limits in animal feedingstuff (European Commission, 2002) and *Regulation (EC) No 333/207* lays down sampling methods and methods of analysis for the official control of the levels of mercury and other restricted substances in foodstuffs (European Commission, 2007).

The European Food Safety Authority (EFSA) and national food safety authorities provide advice on fish consumption in an attempt to minimise mercury intake. According to EFSA's scientific opinion from 2015 (European Food Safety Authority (EFSA), 2015), limiting consumption of fish species with a high methylmercury content is the most effective way to achieve the health benefits of fish whilst minimising the risks posed by excessive exposure to methylmercury.

EFSA recommended that individual Member States, particularly those where fish/seafood species with a high mercury content – such as swordfish, pike, tuna and hake – are consumed regularly, consider their national patterns of fish consumption and assess the risk of different population groups exceeding safe levels of methylmercury while obtaining the health benefits of fish. Earlier EFSA scientific opinions (European Food Safety Authority (EFSA), 2014), (European Food Safety Authority (EFSA), 2012), (European Food Safety Authority (EFSA), 2004) looked respectively at the risks from mercury and methylmercury in food, and the health benefits of fish/seafood.

The first opinion established a TWI for methylmercury of 1.3 micrograms per kg of body weight; the second recommended weekly intakes of fish of between 1-2 servings and 3-4 servings in order to realise health benefits such as improved neurodevelopment in children and reduced risk of coronary heart disease in adults respectively, as was already proved in the DEMOCOPHES project (Castaño, et al., 2015).

In September 2018, the Standing Committee on Plants, Animals, Food and Feed of the European Commission, reported that for the time being, the review of the maximum levels (MLs) for mercury in fish will be discontinued. However, the Commission stressed the importance of consumption advice related to mercury in fish and encouraged Member States to:

- ▶ develop specific national consumption advice related to fish consumption, in order to fully achieve the beneficial effects of fish consumption, whilst limiting the risks of mercury toxicity. When developing this consumption advice, Member States shall especially include the frequency of fish consumption and the fish species consumed;
- ▶ communicate the specific national consumption advice to the consumers as well as to relevant health care workers, working with the consumer groups most at risk.

It further stated that possible data on the effectiveness of consumption advice can be sent to the Commission (European Commission Standing Committee on Plants, 2018).

Several Member States do not have national guidelines for fish/seafood consumption. Some Member States provide food safety advice on fish consumption by pregnant women and young children. Existing national guidelines exhibit great variation in their content, complexity and presentation style. According to a recent review, existing guidelines, are largely based on the mercury content of fish and far less consideration is given to the beneficial effects of nutrients provided by fish. Furthermore, the complexity of the guidelines may lead to pregnant women reducing or eliminating their fish intake, which can have negative consequences on the offspring (Taylor, Emmett, Emond, & Golding, 2018).

Two systematic literature reviews investigated the relationship between seafood consumption during pregnancy and childhood and neurocognitive development. They concluded that there is moderate and consistent evidence indicating that consumption of a wide range of amount amounts

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and types of commercially available seafood during pregnancy is associated with improved neurocognitive development of offspring as compared to eating no seafood. Although the data were insufficient for a conclusive statement regarding neurocognitive effects from types of seafood or specific species, overall, the benefits to neurocognitive development provided by seafood appear to exceed the potential harms from mercury exposure (Hibbeln, et al., 2019). The effect of consumption of a specific species of fish (Atlantic cod, which generally has low levels of methylmercury contamination) on mercury exposure of pregnant women was investigated in a randomised controlled trial in Norway. The results showed that intervening to achieve 400g of cod fillets per week for 16 weeks, slightly increased the total hair mercury in the intervention group, but did not lead to an increase in the number of subjects exceeding the US EPA reference dose (Næss, et al., 2020).

15.1.3.3 Chemicals

Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) restricts specific uses of mercury under Annex XVII and was amended by *Commission Regulation (EC) No 552/2009* to also restrict mercury in measuring devices intended for use by the general public. Annex XVII was further amended by *Commission Regulation (EC) No 847/2012* to restrict mercury-containing measuring devices intended for industrial and professional uses. *Commission Regulation (EU) No. 848/2012* prohibited the manufacture, use and placement on the market of five phenylmercury compounds from 10 October 2017. To date, mercury has three active registrations under REACH (European Chemicals Agency, ECHA).

Mercury has been assigned a European Union harmonised classification and labelling according to *Regulation (EC) No 1272/2008* of the European Parliament and of the Council on classification, labelling and packaging of substances and mixtures (CLP) (European Chemicals Authority (ECHA), n.d.) (see §15.1.1). Mercury and its compounds are included in the “Public Activities Coordination Tool” (PACT) list, which provides up-to-date-information on the activities planned, ongoing or completed by ECHA and or Member States Competent Authorities in the frame of the REACH and CLP regulations (European Chemicals Authority (ECHA), n.d.). Mercury is subject to the “Prior Informed Consent regulation” (PIC, *Regulation (EU) 649/2012*) and to export notification procedure (European Commission, 2012).

15.1.3.4 Environment

Regulation (EU) 2017/852 transposes in the European Union the obligations under the Minamata Convention on Mercury (see §15.1.3.7). It covers the full life cycle of mercury and complements existing EU environmental law on mercury and repeals regulation (EC) No 1102/2008 (European Commission, 2017).

It prohibits the export of mercury and mercury compounds, and the manufacture, export and import of a large range of mercury-added products, restricts all uses of mercury catalysts and large electrodes in industrial processes and future new uses of mercury in industry and in products and requires that all mercury waste is safely taken out of the economic sphere, stabilised in a less toxic form and stored permanently in environmentally sound conditions.

It also sets restrictions on the use of dental amalgam, which is the last large use of mercury in the EU, and sets out a process to assess the feasibility of a complete phase out of the use of mercury in dentistry. As from 1/7/2018, the use of dental amalgam is prohibited for dental treatment of (i) deciduous teeth, (ii) of children under 15 years and (iii) of pregnant or breastfeeding women, unless deemed strictly necessary by the dental practitioner on the ground of specific medical needs of the patient. By 1/7/2019, each Member State must set out and publish on the Internet a

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national plan on measures to phase down the use of dental amalgam. As from 1/1/2019, dental practitioners are no longer allowed to use dental amalgam in bulk, but only in pre-dosed encapsulated form and all dental facilities using amalgam and/or removing dental amalgam fillings must be equipped with amalgam separators ensuring the retention and collection of amalgam particles with a view to preventing their release into wastewater systems. Dental practitioners must ensure that their amalgam waste is handled and collected by authorised waste management establishments or undertakings (no direct or indirect release into the environment).

The Commission shall report by 30/6/2020 on the feasibility of a phase out of the use of dental amalgam in the long term, and preferably by 2030, and present concomitantly, if deemed appropriate, a legislative proposal.

The EU Water Framework Directive (“WFD”, *Directive 2000/60/EC*) requires EU Member States to ensure that water bodies achieve good chemical and ecological status. Directive 2013/39/EU sets environmental quality standards for mercury in surface waters and fish to protect higher level predators from secondary poisoning through bioaccumulation. The Groundwater *Directive 2006/118/EC*, the Environmental Quality Standards *Directive 2008/105/EC* and the Dangerous substances *Directive 2006/11/EC* complement the overall framework for integrated management. In particular *Decision 2455/2001/EC* (which forms Annex X of the Water Framework Directive) establishes the list of priority substances and priority hazardous substances for which measures must be adopted. *Directive 2006/118/EC* also complements the provisions preventing or limiting inputs of pollutants into groundwater already contained in the WFD. According to the European Environment Agency (European Environment Agency, 2018), ~41% of surface water bodies in the EU exceed the mercury concentration for protecting fish-eating birds and mammals.

Directive 2010/74/EU lays down rules on integrated prevention and control of pollution arising from industrial activities and rules designed to prevent or, where that is not practicable, to reduce emissions into air, water and land and to prevent the generation of waste, in order to achieve a high level of protection of the environment taken as a whole. This includes mercury and its compounds, expressed as mercury (Hg).

The Waste Incineration *Directive 2000/76/EC* aims to prevent or to limit pollution from the incineration and co-incineration of waste requiring operators of plants with a nominal capacity of 2 tonnes or more per hour to provide the competent authority with an annual report including emissions into air and water, but there is no specific requirement for an emission inventory. Member States provide reports to the Commission on implementation progress based on questionnaire sent by the Commission to Member States every three years. Periodic measurement is required but no obligation for an annual inventory is specified.

15.1.3.5 Consumer products

Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products prohibits mercury in cosmetic products (Annex II). Limited exemptions for mercury compounds used as preservatives in cosmetics are provided in Annex V.

The Restriction of Hazardous Substances *Directive 2002/95/EC* bans the use of mercury in Electrical and electronic equipment.

Directive 2008/12/EC in conjunction with *Directive 2006/66/EC* restricts mercury in batteries and accumulators.

15.1.3.6 Occupational health and safety

Chemical Agents *Directive 98/24/EC* lays down minimum requirements for the protection of workers from risks to their safety and health arising, or likely to arise, from the effects of chemical

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agents that are present at the workplace or as a result of any work activity involving chemical agents. *Directive 2009/161/EU* established a third list of indicative occupational exposure limit values (IOELVs), which includes an IOELV for mercury and divalent inorganic mercury compounds for the protection of workers who may be exposed to mercury. Member States may have regulated the exposure limit value for alkyl compounds of mercury (e.g. Spain, 0.01 mg/m³).

15.1.3.7 Global Policy

The Minamata Convention on Mercury

The *Minamata Convention on Mercury* is a global treaty, effective as of 16 August 2017, which aims to protect human health and the environment from anthropogenic emissions and releases of mercury and mercury compounds. It has been ratified by 99 parties, including the European Union. The obligations under the Convention are transposed in the EU by Regulation (EU) 2017/852 on mercury.

Some issues covered by the Convention, which relate to the scope of HBM4EU, are:

- ▶ Capacity-building, technical assistance and technology transfer
- ▶ It calls for cooperation between Parties for timely and appropriate capacity-building and technical assistance to developing country Parties.
- ▶ Health aspects
- ▶ It encourages Parties to promote the development and implementation of strategies and programmes to identify and protect populations at risk, to promote appropriate health-care services for prevention, treatment and care for populations affected by the exposure to mercury or mercury compounds and to establish and strengthen institutional and health professional capacities.
- ▶ Information exchange
- ▶ It calls for exchange of information concerning mercury and mercury compounds, including toxicological and safety information, and of epidemiological information concerning health impacts associated with exposure to mercury and mercury compounds, in close cooperation with the World Health Organization and other relevant organisations, as appropriate.
- ▶ Public information, awareness and education
- ▶ It calls for the provision to the public of available information, awareness and education about the effects of exposure to mercury/mercury compounds on human health/environment, about alternatives and about results from research & monitoring activities

Research, development and monitoring

It calls for Parties to cooperate to develop harmonised methodologies and to use them within their capacity, for modelling and geographically representative monitoring of levels of mercury and mercury compounds in vulnerable populations, for collaboration in the collection and exchange of relevant and appropriate samples and for assessments of the impact of mercury and mercury compounds on human health and the environment.

- ▶ Reporting
- ▶ Each Party shall report to the Conference of the Parties (COP) on the measures it has taken to implement the provisions of the Convention, on the effectiveness of such measures and of possible challenges in meeting the obligations of the Convention.
- ▶ Effectiveness evaluation
- ▶ The effectiveness of the Convention will be evaluated by COP within six years from the date of entry into force of the Convention and periodically thereafter, using comparable monitoring data on the presence and movement of mercury and mercury compounds in the environment

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as well as trends in levels of mercury and mercury compounds observed in biotic media and vulnerable populations.

15.1.4 Technical aspects

15.1.4.1 Availability of biomarkers and methods

Establishing a quantitative dose-response relationship is particularly challenging for mercury because it can exist in different forms (elemental mercury, mono- and divalent mercury and organic mercury), each having different kinetic properties (Ha, et al., 2017).

Mercury concentrations can be measured in different human matrices: hair, urine, blood, nails, breast milk, cord tissues, cord blood and the placenta. The choice of matrix depends on the time of sampling after exposure, if chronic or acute exposure will be investigated and the type of mercury compounds, which will be assessed (Miklavčič Višnjevec, Kocman, & Horvat, Human mercury exposure and effects in Europe, 2014). Other “unconventional” matrices may be used, depending on the study objectives and design.

- ▶ **Hair:** is a non-invasive matrix that is easy to sample and analyse and is very useful for monitoring long-term methylmercury exposure in the general population. Methylmercury analysis in other matrices requires complicated, time-consuming and expensive methods and so has very limited use in large Human Biomonitoring surveys. Both inorganic and organic forms of mercury bind to the hair structure, but there is a strong preference for MeHg. Methylmercury is incorporated into the follicle during hair formation. Once transported by the blood into follicular cells, it binds to cysteines of keratin proteins and it constitutes approximately 80% or more of the total mercury in hair for fish-consuming populations (National Research Council, Committee on the Toxicological Effects of Methylmercury, Board on Environmental Studies and Toxicology, 2000). The concentration of total mercury in scalp hair is proportional to the simultaneous concentration in blood, but in the case of exposure to methylmercury, it is ~250 times higher. Hair-to-blood concentration ratios of methylmercury can be highly variable among individuals. The error in blood Hg estimated from hair Hg using the WHO recommended hair-to-blood ratio of 250 was evaluated by Liberda et al. (2014) and it ranged -25% to +24%, with systematic underestimation for females and overestimation for males (Liberda, et al., 2014). Assuming a growth rate of 1.1 cm/month for scalp hair, an indication of temporal exposure is provided, but the uncertainty associated with this assumption must be considered (World Health Organization (WHO), 2010), (Sakamoto, et al., 2004). Hair mercury concentrations can be affected by several factors, including hair colour and variable growth rates, which can limit its usefulness as an indicator of Hg concentrations in the body (Miklavčič Višnjevec, Kocman, & Horvat, Human mercury exposure and effects in Europe, 2014). Quality assurance and control systems are required for accurate results (e.g. possible external contamination) (Grandjean, Jørgensen, & Weihe, Validity of mercury exposure biomarkers, 2002). QA/QC measures were already defined and tested in DEMOCOPHES with good results. These measures include sampling SOPs, training (including a video for hair sampling (ISCI)) and ICI/EQUAS for mercury analysis in hair (Esteban, et al., 2015). Recently, the World Health Organization published standard operating procedures for the assessment of mercury in hair, cord blood and urine, with emphasis on quality control as a prerequisite for getting reliable results. This report also provides information on alternative methods that can be used for analysis of mercury (World Health Organization, 2018).
- ▶ **Blood:** in children and adults, can be used to assess short-term (~1 week) exposure. It involves invasive sampling and storage / transportation require attention. Speciation

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analysis is preferable for a comprehensive assessment of the type and magnitude of the exposure. Recently, dried blood spot (DBS) samples were demonstrated to be a useful matrix for assessment of mercury and methylmercury exposure. This development may be especially useful for the assessment of the exposure of new-borns (Basu, et al., 2017), (Santa-Rios, Barst, & Basu, 2020).

- ▶ **Urine:** The predominant form in urine is inorganic mercury and so total urinary mercury reflects the internal dose of the inorganic form. Urine is a suitable biomarker of long-term low-exposure to both inorganic and elemental Hg, because it contains Hg which accumulated in the renal tissue (i.e., kidney is the target organ) during a chronic exposure (Ruggieri, Majorani, Domanico, & Alimonti, 2017), (Miklavčič Višnjevčec, Kocman, & Horvat, Human mercury exposure and effects in Europe, 2014), (INRS).
- ▶ **Cord-blood:** is the most desirable biomarker for estimating pre-natal exposure. Total Hg in cord blood estimates foetal exposure over a longer period than that provided by maternal blood and provides a better indication of the risk for developmental neurotoxicity. However, it does not provide information on exposure variability during gestation and its storage and transportation are more complicated (Ruggieri, Majorani, Domanico, & Alimonti, 2017).
- ▶ **Umbilical cord tissue:** is a useful matrix for assessment of foetal middle-term exposure, sampling is simple and it is non-invasive. Total Hg represents exposure during the third trimester, but doesn't provide information on sensitive short-term variation. A dry weight-based total Hg concentration is more accurate, but more labor-intensive (Ruggieri, Majorani, Domanico, & Alimonti, 2017).
- ▶ **Nails:** Maternal mercury concentrations in nails at parturition have also been shown to have a strong correlation with mercury concentration in cord blood and can be used as biomarker (Ha, et al., 2017). Generally, this matrix assesses long-term (chronic) exposure. Sampling is simple, non-invasive and easy to preserve. Quality assurance/quality control systems are required for accurate results. Fingernails are sometimes contaminated (Ruggieri, Majorani, Domanico, & Alimonti, 2017).
- ▶ **Breast milk:** is useful for investigation of long-term exposure. Total Hg is suitable for estimating maternal exposure and for predicting the potential exposure for breast-feeding in infants (Ruggieri, Majorani, Domanico, & Alimonti, 2017).
- ▶ **Cerebrospinal fluid / Brain:** The use of such “unconventional” matrices, in combination with speciation analysis, can be useful for the investigation of the neurotoxic effects on the target system / organs. So far, there have been only few applications of this approach, due to limited access to cerebrospinal fluid and brain samples, analytical challenges caused by matrix interferences, low concentrations and limited stability of many trace element species of interest. Modern, powerful analytical techniques, which provide advanced validity and chemical information are necessary (Michalke, Willkommen, Drobyshev, & Solovyev, 2018), (Michalke, Halbach, & Nischwitz, JEM Spotlight: Metal speciation related to neurotoxicity in humans, 2009).
- ▶ **Meconium.** The earliest stool of new-borns (meconium), which is composed of materials ingested in utero, has been demonstrated to be a suitable matrix for prenatal mercury and methylmercury exposure assessment (Trdin, et al., 2019).

The determination of mercury in biological specimens requires sensitive analytical methods, performed under good quality control conditions. The DEMOCOPHES experience proved that is possible to study the exposure to mercury in a harmonised way if common Standard Operating Procedures (SOPs) are applied and under a Quality Assurance / Quality Control (QA/QC) scheme (Esteban, et al., 2015). Various methods exist that differ in sample preparation technique and/or

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the detections system. Determination of total Hg concentration can be done by (1) acid digestion followed by cold vapour atomic absorption technique (CV AAS), cold vapour atomic fluorescence (CV AFS) and/or ICP MS detection; (2) thermal combustion of a sample, gold amalgamation and AAS detection.

Speciation of mercury requires complex and lengthily analytical procedures and expensive reagents and equipment, which are not routinely available in analytical laboratories. Speciation analysis is necessary to differentiate between inorganic/elemental and methyl mercury exposure. It may be possible to obtain information without the need of speciation, by using a combination of different matrices, the choice of which should depend on the type of the hypothesised exposure.

15.1.4.2 Need for new approaches

Despite the plethora of data on exposure to mercury, the results are fragmented because different studies use different approaches, which limit their usefulness. It is important to harmonise the approaches used to investigate different study populations. The DEMO/COPHES (Esteban, et al., 2015) and the pilot UNEP/WHO project on mercury biomonitoring (World Health Organization, 2018) have laid the basis for harmonisation of exposure biomarkers, which needs to be further advanced (Ha, et al., 2017). HBM4EU provides a golden opportunity to improve on this basis, to test it in additional countries and to use to for answering specific policy questions.

The selection of best-suited matrices and biomarkers of exposure is crucial. For example, if hypotheses on the effects of MeHg exposure on child development will be tested, the best suited matrices and biomarkers of foetal exposure to MeHg should be selected.

The development of simple, robust and cost- effective methods for measuring total and organic mercury simultaneously is very important.

Development of suitable dietary advice for fish consumption for vulnerable groups, such as pregnant women and young children, is a major public health objective. At present, some countries have national guidelines, while many others do not. There is significant variability in existing guidelines, how they are developed and how they are communicated to stakeholders (Taylor, Emmett, Emond, & Golding, 2018). The formulation of dietary advice needs to be based on a benefit/risk analysis, considering the nutritional benefits of fish as a whole food vs. the risk of exposure to mercury and other contaminants. Further work is necessary to better understand the factors coming into play in this analysis (e.g. standardisation of outcome measures in cohorts involving assessment of exposure to mercury, the contribution of nutrients / mercury exposure of different fish species consumed, their origin and method of preparation, randomised controlled trials to better support causal interferences, genetic variants etc) (Hibbeln, et al., 2019).

Markers of susceptibility need to be validated (Karagas, et al., 2012). These are important for understanding the human health effects of low-level MeHg exposure as a basis for future research efforts, risk assessment, and exposure remediation policies worldwide (Karagas, et al., 2012). Hg speciation in biological matrices, particularly blood, would provide characterisation of species-specific exposure at levels relevant for European population. Individuals' inherited factors seem to play a role in determining toxic effects of environmental contaminants, including those of mercury. In recent years interest in gene-environment interaction has grown substantially, because of the progress in laboratory techniques, improved understanding of genetics and realisation of complex mechanisms between genetics and environment (Basu, Goodrich, & Head, Ecogenetics of mercury: from genetic polymorphisms and epigenetics to risk assessment and decision-making, 2014), (Andreoli & Sprovieri, 2017). Identification and validation of novel biomarkers of susceptibility is therefore an important part in investigation of exposure-health relationships.

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Research on the elimination and enhancement of excretion of mercury is also needed and is important for risk management options.

15.1.5 Societal concern

Societal concern regarding mercury is very high.

Mercury is considered by WHO as one of the top ten chemicals or groups of chemicals of major public health concern (World Health Organization (WHO), n.d.).

European citizens consider environmental pollution as the top risk most likely to affect them personally, according to Special Eurobarometer 238 on risk issues. Although people do not differentiate greatly between the various types of risks, they are more likely to worry about risks caused by external factors over which they have no control. Mercury was reported as one of the top risks they are concerned about. In almost all Member States, at least one citizen in two is worried about pollutants like mercury or dioxins (European Commission, 2006). According to Special Eurobarometer 354 on food-related risks, one third of Europeans are very worried about mercury in fish (European Commission, 2010). This concern is validated by the fact that in 2017, mercury in fish was the second most notified hazard in RASFF for exceedance of the maximum limit set in EU legislation (European Commission, 2018).

Due to its classification as a substance toxic to reproduction (“CRM” according to Annex VI of Regulation 1272/2008) (Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures), mercury is included in the “SIN (Substitute It Now!) List”, a comprehensive database of chemicals likely to be restricted or banned in the EU developed by the non-governmental organisation “International Chemical Secretariat” (ChemSec).

Mercury ranks 3rd and methylmercury 116th out of 275, on the “Substance Priority List” (SPL) prepared biannually by the ATSDR for substances most commonly found at facilities on the National Priorities List (NPL) and which are determined to pose the most significant potential threat to human health due to their known or suspected toxicity and potential for human exposure (US Agency for Toxic Substances and Disease Registry (ATSDR), 2017).

Mercury and its organic compounds are included in the “OSPAR List of Chemicals for Priority Action” of the OSPAR convention for the protection of the marine environment of the North-East Atlantic (OSPAR Convention for the protection of the marine environment of the North-East Atlantic, n.d.).

Several European and global non-governmental organisations recognise mercury pollution as a top priority, which must be addressed. Examples include:

- ▶ “Zero Mercury” campaign of the European Environmental Bureau (EEB) (European Environmental Bureau (EEB), n.d.).
- ▶ The EEB is the largest network of environmental citizens’ organisations in Europe, with around 140 member-organisations in more than 30 countries (including all EU Member States) and representing 30 million individual members and supporters.
- ▶ “Mercury-Free” campaign of IPEN (IPEN, n.d.).
- ▶ IPEN is a global network of public-interest NGOs, comprising of over 500 participating organisations in more than 100 countries
- ▶ “Zero Mercury” campaign of the Zero Mercury Working Group (ZMWG) (Zero Mercury Working Group (ZMWG) , n.d.).

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- ▶ The Zero Mercury Working Group (ZMWG) is an international coalition of over 95 public-interest environmental and health non-governmental organisations from more than 50 countries.
- ▶ “Stay Healthy, Stop Mercury” campaign, of the Health and Environment Alliance (HEAL) (Health and Environment Alliance).
- ▶ HEAL is a not-for-profit organisation addressing how the natural and built environments affect health in the European Union (EU).

Mercury and its compounds were voted by stakeholders who participated in the Stakeholder Workshop organised in the frame of HBM4EU in on November 20th 2017 as a “top substance of concern” and ranked in the 4th position. Stakeholders expressed concern regarding exposure from fish consumption (with pregnant women mentioned as an especially vulnerable group) and about the effects of lifelong exposures from multiple pathways. Mercury is a highly regulated substance but there is fragmentation into different pieces of legislation, which are not presently aligned. Stakeholders expressed the need for traceability, coordination, alignment and integration of data and policy. They also advocated that information on exposure levels should be made available and the exposure of the total population and specific exposure groups should be compared. Stakeholders would use the result of HBM4EU for a comparison of reference values for the general population to exposure of workers and for communication to citizens. They stated that within HBM4EU, information should be collected and made available in one single database. They also see a need for interpretation results for the purpose of generating and communicating useable advice for the public in an understandable manner.

15.2 Categorisation of Substances

The proposed category for Mercury is Category A.

The health impact of mercury is well documented and the European Commission introduced policies to manage the risk, e.g. restriction of use in industry, regulatory limit values in food. Data on total mercury exposure from different countries across Europe are available. However, several countries lack recent data or data on vulnerable populations, such as children. Also, in most instances, sampling is not representative of the population.

The proposed category for Methylmercury is Category B.

The health impact of methylmercury is well documented. Data on methylmercury exposure in Europe is not as common as for total mercury. Since hair mercury is mostly in the form of methylmercury, results on the concentration of mercury in hair provide a good indication of exposure to methylmercury. Representative data on the geographic spread of exposure and association with specific sources of exposure (e.g. associations with specific species of fish) are missing in Europe.

Some recommendations have been proposed by Food Safety Authorities in order to reduce methylmercury exposure through seafood in Europe, but a harmonised European global policy on this substance is lacking.

These recommendations have been based on studies of populations with unique diets. Further investigation is needed to understand the risks associated with typical diets in Europe.

The effects of chronic exposure to low levels and the factors of susceptibility have not been adequately investigated.

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Table 15-7: Substances included in the substance group, listed according to availability of toxicology and human biomarker data, in category A, B, C, D, E substances (see general introduction)

Category	Abbrev./Acronym	Systematic name	CAS No.	Regulation
A	Hg	Mercury	7439-97-6	See §0*
B	MeHg, CH ₃ -Hg	Methylmercury	22967-92-6	See §0*

* Section §0 provides an overview of relevant policies. Most policies refer to “mercury” or “mercury and its compounds” or “total mercury” and do not discriminate among the different forms of mercury.

15.3 Policy-related questions

Section 15.1 presents an overview of current EU policies related to mercury, including the Minamata Convention, a global treaty to address mercury pollution, which was ratified by the EU.

The following policy-related questions relate to commitments under this frame:

1. How effective are policy actions to reduce human exposure to mercury in Europe? (including the EU’s Strategy on Mercury and the Minamata Convention, which was ratified by the EU and Member States)?
2. How can harmonised, validated and comparable information be collected and transferred to support and evaluate current policies?
3. What biomonitoring and exposure data on mercury (and its species), relevant to the European population, are currently available and what new data are needed to address policy-related questions?
4. What is the geographic spread of the current exposure and how does it relate to different exposure sources (environmental; contaminated sites; dental amalgams; dietary, including different species of sea-food)? Ideally, this should capture the exposure of highly exposed populations (e.g. high seafood consumers with distinction of populations consuming predator fish from those with low/no consumption of such fish, such as Southern & Northern Europeans, European arctic populations), but also of low-exposure populations for comparison.
5. Which populations remain vulnerable to health impacts from mercury exposure and how can they be protected?
6. How can the public be informed and how can public awareness and education be raised regarding the effects of mercury on health and the environment and about management options?

What advice should be given regarding dietary recommendations to vulnerable Europeans (e.g. pregnant women, infants, high sea-food consumers) and other stakeholders (e.g. health practitioners, policy makers) to reduce exposure to mercury while in keeping with nutritional requirements and cultural dietary preferences? Ideally, this should consider the different types of foodstuff (e.g. types of seafood) consumed in different parts of the EU, the toxicity and occurrence of the different mercury species in different foodstuff and the positive effects of n-3 long-chain polyunsaturated fatty acids in fish and of micro nutrients (e.g. selenium) in the diet.

Related to this, how can HBM4EU results support policy decisions at EFSA and ECHA?

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7. At what level of exposure to different mercury species and to total mercury are health effects likely to occur? Current guidance values were based studies of the Faroese people, who have a diet that is unique and does not relate to food consumption patterns in the EU. This important issue has not been given proper attention to date.

8. How does exposure relate to the manifestation of adverse health effects?

What are possible health effects resulting from chronic low exposure to mercury and its organic compounds (such as from food consumption and dental amalgams)? This type of exposure is the most relevant for Europeans and can be addressed by speciation analysis of biobanked samples from existing cohorts and associations with adverse health effects.

What factors make people more susceptible to the development of health effects due to mercury exposure?

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15.4 Research Activities to be undertaken

Table 15-8: Listing of research activities to be carried out to answer the policy questions

Policy question (PQ)	Substance	Available knowledge	Knowledge gaps and activities needed
PQ1. How effective are policy actions to reduce human exposure to mercury in Europe? (including the EU's Strategy on Mercury and the Minamata Convention, which was ratified by the EU and Member States)?	Mercury and methylmercury	See chapter 0	<p>WP 2,4,5,6,7,8,9,10,11,12,13,14 - Overarching activity: Support the establishment of permanent European mercury biomonitoring as long-term support of global mercury policies. Emphasis on transfer of knowledge to enable new, quality-assured, comparable data and their interpretation in countries which ratified the Minamata Convention through the established procedures at EU level. This is also relevant to PQs 2,3,4.</p> <p>Development of tools, collection of relevant data to assess the exposure of Europeans to mercury and methylmercury, harmonised analysis to assess exposure and its determinants and to facilitate assessment of time trends, making data and results available to policy makers and other stakeholders.</p> <p>WP 2,7,8,9,10,12: Implementation of an aligned intervention study aiming at controlling prenatal exposure to mercury in high fish-consuming countries (proposition under evaluation by the Management Board). Also relevant to PQ2,4,5.</p>
PQ2. How can harmonised, validated and comparable information be collected and transferred to support and evaluate current policies?	Mercury and methylmercury	See chapters 15.1.4 and 15.1.2	<p>WP7: Generation and distribution of survey via National Hubs, aiming to collect information on recent (past 5 years), ongoing and planned studies, which include mercury biomonitoring. Generation of tools for harmonised and quality-assured recruitment, sampling, sample storage and transfer, questionnaires, communication materials.</p> <p>WP9: Development and update (as information becomes available), of inventories and evaluations of the best exposure biomarkers, matrices and analytical methods relevant to mercury biomonitoring. Also relevant to PQs 1,3,4,5. Development and update (as information becomes available), of the inventories of candidate laboratories for the analysis of biological samples for mercury biomonitoring. Also relevant to PQ3.</p> <p>WP10: Development of guidelines and tools for harmonised data transfer, storage and statistical analysis. Making data accessible to policy managers and other stakeholders on IPChem to the extent possible.</p> <p>WP11: Development of guidelines to help standardisation of measurements and comparability of collected health data relevant to mercury, in future studies. Also relevant to PQ6.</p>

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Policy question (PQ)	Substance	Available knowledge	Knowledge gaps and activities needed
PQ3. What biomonitoring and exposure data on mercury (and its species), relevant to the European population, are currently available and what new data are needed to address policy-related questions?	Mercury and methylmercury	See chapter 15.1.2.3	WP7 (and as relevant, WPs 8, 10,12,13,14) Identification and systematic collection of relevant recent or ongoing European studies, identification of knowledge gaps, prioritisation of research needs. Review and analysis of existing epidemiological and toxicological data on mercury and its species as needed to address policy questions.
PQ4. What is the geographic spread of the current exposure and how does it relate to different exposure sources (environmental; contaminated sites; dental amalgams; dietary, including different species of sea-food)? Ideally, this should capture the exposure of highly exposed populations (e.g. high seafood consumers with distinction of populations consuming predator fish from those with low/no consumption of such fish, such as Southern & Northern Europeans, European arctic populations), but also of low-exposure populations for comparison. Which populations remain vulnerable to health impacts from mercury exposure and how can they be protected?	Mercury and methylmercury	See chapters 0, 15.1.5, 15.1.2.2 and 15.1.2.3	WP10 (other WPs e.g. 5, 7, 8, 12 and possibly others, may also be involved as relevant) Collection, integration and making available existing HBM data on mercury into IPChem. Also relevant to PQ1,2,3. Analysis to the extent possible of existing & available HBM data to assess (a) baseline exposure of Europeans to organic / total mercury and the associated risk and to facilitate the assessment of temporal trends with regards to the effectiveness of policies, (b) determinants of exposure, including geographic variations and their causes (e.g. environmental exposures, diet), (c) generation of European reference values for mercury exposure, (d) identification of groups at risk of exceeding health-based guidance values (e.g. by age, gender, highly exposed, hot-spots in Europe). This is a core activity. Also relevant to PQs1,2,3. WP12: Optimise the integrated exposure modelling platform by updating thof the exposure model parameterisatoin for mercury using available data; Characterisation of the toxicokinetic behavior differences in internal dose of mercury species.; identification of internal exposure to different mercury species and to tal mercury for various age groups; definition of optimised sampling schemes.

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Policy question (PQ)	Substance	Available knowledge	Knowledge gaps and activities needed
<p>PQ5. How can the public be informed and how can public awareness and education be raised regarding the effects of mercury on health and the environment and about management options?</p> <p>What advice should be given regarding dietary recommendations to vulnerable Europeans (e.g. pregnant women, infants, high sea-food consumers) and other stakeholders (e.g. health practitioners, policy makers) to reduce exposure to mercury while in keeping with nutritional requirements and cultural dietary preferences? Ideally, this should consider the different types of foodstuff (e.g. types of seafood) consumed in different parts of the EU, the toxicity and occurrence of the different mercury species in different foodstuff and the positive effects of n-3 long-chain polyunsaturated fatty acids in fish and of micro nutrients (e.g. selenium) in the diet.</p> <p>Related to this, how can HBM4EU results support policy decisions at EFSA and ECHA?</p>	Mercury and methylmercury	See chapters 15.1.1.2, 15.1.3.2, 15.1.5 and 15.1.3.3	<p>WP2 and as relevant WP4,5,6,7,8,9,10,11,12,13,14</p> <p>Collection, curation and provision of information relevant to the mercury chemical group (CG) as it becomes available (e.g. results, targeted communication products – including dietary advice to the extent feasible & relevant, common methods, protocols), to targeted audiences (e.g. public, health practitioners, scientists, policy makers) via the Knowledge Hub.</p> <p>WP4: Mapping of the information needs of external bodies related to mercury (e.g. understanding the perspectives of the public through focus groups).</p> <p>WP5: Reporting on progress achieved by HBM4EU for the mercury CG. Establishment of HBM-based guidance values for mercury for the general population provided that sufficient epidemiological / toxicological / toxicokinetic data re available. If not, provide recommendations for data needed to fill the gap. Also relevant to PQ1,4.</p> <p>Development of a proposal on how to integrate HBM in risk assessment procedures and use of available mercury HBM data for risk assessment. Also relevant to PQ1,4.</p> <p>Based on the availability of aggregated data, construction of HBM-based indicators for mercury and development of associated information to facilitate their interpretation by stakeholders, including policy makers. Also relevant to PQ1,4.</p> <p>WP11: Development of scoping reviews for health professionals, on the association of environmental exposures to manifestation of specific non-communicable diseases.</p> <p>WP13 and as relevant WP5: Through a critical review of the literature published since EFSA's 2012 risk assessment, determine if recent findings on the health effects of mercury are consistent with the previously assessed evidence.</p>

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Policy question (PQ)	Substance	Available knowledge	Knowledge gaps and activities needed
PQ6. At what level of exposure to different mercury species and to total mercury are health effects likely to occur? Current guidance values were based studies of the Faroese people, who have a diet that is unique and does not relate to food consumption patterns in the EU. This important issue has not been given proper attention to date.	Mercury and methylmercury	See sections § 15.1.2 (15.1.2.4), 0 (15.1.3.7)	<p>WP11: Development of guidelines to help standardisation of measurements and comparability of collected health data relevant to mercury, in future studies. Also relevant to PQ1.</p> <p>WP12: Depending on data availability on total mercury and/or mercury species, use of exposure modelling to explore the linking of internal exposure to external sources for vulnerable population groups, investigation of substance toxicological behaviour, risk characterisation, support of the evaluation of the effectiveness of existing regulatory frames. This is also relevant to PQs 1,4,5,7</p>

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Policy question (PQ)	Substance	Available knowledge	Knowledge gaps and activities needed
<p>PQ7. How does exposure relate to the manifestation of adverse health effects?</p> <p>- What are possible health effects resulting from chronic low exposure to mercury and its organic compounds (such as from food consumption and dental amalgams)? This type of exposure is the most relevant for Europeans and can be addressed by speciation analysis of biobanked samples from existing cohorts and associations with adverse health effects.</p> <p>- What factors make people more susceptible to the development of health effects due to mercury exposure?</p>	Mercury and methylmercury	See chapters 15.1.1, 15.1.2.3 and 15.1.3.7	<p>WP13, 10</p> <p>Investigate allele frequencies of relevant Single Nucleotide Polymorphisms (SNPs) across Europe and how this might contradict exposure/health found so far, since inherited factors of individuals seem to play a role in determining toxic effects of mercury. Also relevant to PQ6.</p> <p>WP13</p> <p>Use the available Mediterranean cohort to (a) examine the impact of mercury on neurobehavior while taking into account co-exposure to other neurotoxic contaminants and to beneficial elements and (b) explore relevant genetic polymorphisms. Also relevant to PQ6.</p> <p>Investigation of the causal pathways from exposure to mercury to health outcomes (Adverse Outcome Pathways)</p> <p>Apply automated text mining tools (e.g. AOP help-finder and systems biology approach to explore exposure/health associations and to promote broader use of mechanistic toxicology information and AOP among risk assessors.</p> <p>WP14</p> <p>Identify the most suitable biomarkers of effect for mercury through a focused literature search of mercury-related human studies and reported health endpoints. Also relevant to PQ2.</p> <p>By integrating all information obtained on effect biomarkers related to HBM4EU priority substances, (a) create maps of the most commonly affected physiological pathways affected (e.g. neurodevelopment), (b) establish a holistic framework for connecting epidemiological and toxicological data (focused on effect biomarkers and AOPs) for utility in a future sustainable European HBM agenda. Also relevant to PQ1, 2.</p>

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16 Prioritised substance group: Mixtures

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The phenomenon of mixtures (in the context of HBM) refers to the common occurrence of chemical xenobiotic substances in the body. There is no broadly accepted operational definition of mixtures. In principle, every single substance, once it enters the body, will exhibit its health effects in interaction with a person's genetic makeup and acquired characteristics, and in concert with all other (xenobiotic) substances from previous and simultaneous exposures. These combined and/or simultaneous may come involuntarily or voluntary through different exposures routes from ambient environments, indoor and occupational environments, food, food additives, consumer products, medication, (medical or voluntary) implants, recreational drugs, performance enhancing drugs and food supplements, tattoo ink, etcetera. These mixtures thus form a challenge to (experimental and observational) science, to scientific assessment of risks and to regulation of substances and general risk management policies. The HBM4EU project addresses how HBM data can contribute to both the science and policy/regulation of dealing with the phenomenon of mixtures. Within the HBM4EU project, the focus for chemical mixtures will be on chemicals with exposure routes through the environment, food, occupation and/or consumer products.

The proposed activities on mixtures in HBM4EU were developed by a working group of experts from the member states. This comprised a first inventory in member states of available data, a preliminary inventory of policy needs in EC institutions combined with a preliminary inventory of specific policy needs in member states, a discussion at the Workshop HBM4EU Proposal Development (16-17th of November 2015, Utrecht), a EEA Workshop Activities on Mixtures under the European Human Biomonitoring Initiative (11th February 2016). In the latter, experts and policy makers jointly outlined the challenges that mixtures pose to science and policymaking. The proposed activities on mixtures in HBM4EU were further developed through e-mail exchanges, with periodic presentations to the HBM4EU Steering Group Meetings. The activities have been grouped into various tasks under WP15. Since the start of the HBM4EU project, these tasks have been refined and/or modified where deemed necessary, based on discussions in dedicated WP15 workshops (November 2017, Utrecht; May 2018, Lisbon; November 2018, Paris; July 2019, Berlin).

Hazardous properties

Since a wide range of chemical substances comprise the mixture of chemical substances in the body, and metabolites thereof, all classes of hazardous properties are potentially involved. This poses the challenge to identify where antagonism, addition or synergies in effects come into play, based on the mode(s) of action underlying adverse health effects.

Dealing with mixtures in research poses specific challenges e.g. (Kortenkamp 2007, Slama 2015). In toxicological research, the mode(s) of action of the different chemicals included in a mixture can be studied in more detail, but typically only a few permutations of possible mixtures can be assessed. This does not do justice to the wide array of substance to what populations are exposed to. On the other hand, observational studies in humans may capture these multiple substances, but

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often fall short in characterising the dynamics of exposure and ADME characteristics (absorption, distribution, metabolism, and excretion) and typically cannot document the modes of action involved and the causality between exposure and observed adverse health effects. Developments in modern techniques such as in sensor technologies, and in epigenomics, transcriptomics, metabolomics, as well as development in biostatistics now allow more in depth research of multiple exposures, body burdens and their effects in humans e.g. (Woodruff 2011, Lenters 2015, Agier 2016). To optimally benefit from these developments new forms of cooperation between traditionally separated research communities and projects need to be built. Based on discussions held in the WP15 workshop in May 2018, five case studies were developed to explore how existing methods can be best applied for human health risk assessment of chemical mixtures and how these methods may inform biomonitoring strategies. The case studies are as follows:

- ▶ Developmental neurotoxicity beyond polybrominated diphenylethers
- ▶ Heavy metals and nephrotoxicity
- ▶ Anti-androgenic chemicals and male reproductive health
- ▶ Chromium (VI), nickel and polycyclic aromatic hydrocarbons and lung cancer
- ▶ Differential exposure misclassification in HBM mixture data due to differences in half-lives between substance

Thus far, the case studies have resulted in an advanced decision tree and workflow scheme for the hazard assessment of chemical mixtures, including decision rules to define when analyses should be refined or discontinued, and adopting clear criteria for the grouping of chemicals to be subjected to mixture risk analyses. These grouping criteria are based on Adverse Outcome Pathway considerations. The lessons learned from the five case studies will comprise relevant insights into the possibilities and limitations of existing methods for human health risk assessment of chemical mixtures, and provide valuable input to recommendations for policy-making and further research.

Exposure characteristics

A central problem in the discussion on mixtures is the virtual absence of adequate exposure data. In many HBM projects, as well as in cohort studies and biobank studies, multiple (groups) of pollutants have been studied; yet the reporting is typically restricted to distributions and central tendency measures of single compounds or groups of compounds. The groups are often clustered on:

- ▶ chemical families, e.g. phthalates, bisphenols, dioxins, PCB's, PAH's, VOC's
- ▶ exposure routes, e.g. food, household dust
- ▶ type application such as plasticisers, flame retardants, pesticides
- ▶ supposed working mechanisms e.g. endocrine disruptors, carcinogens, neurotoxins.

In few cases, the distribution of a measure/indicator of cumulative body burdens in individuals is reported. If so, this only summarises body burdens within the clusters mentioned above and hardly ever overarching indicators are used and reported.

Thus, it is largely unknown whether specific profiles of high exposures exist, i.e. individuals high in PCB's are also in pesticides, flame retardants or poly fluorinated compounds or mycotoxins. Meaningful indicators to capture such profiles need to be developed for mixtures in the wider meaning of the word. With such aggregated mixture indicators exposure profiles of concern and

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potential hotspots or risk groups can then be identified in existing data and in new studies. Therefore, also existing data merit re-evaluation from a mixture perspective.

Policy relevance

Dealing with mixtures poses substantial regulatory challenges, with numerous pertinent EU and national regulations.

In the European Directive 396/2005 EFSA was appointed to be responsible for establishing the methodology for risk assessment of mixtures. It states among other things “...*It is also important to carry out further work to develop a methodology to take into account cumulative and synergistic effects. In view of human exposure to combinations of active substances and their cumulative and possible aggregate and synergistic effects on human health, MRLs should be set after consultation of the European Food Safety Authority...*”. Since 2005 EFSA has published four Opinions and one Guidance on how to perform risk assessment for pesticide mixtures. The full methodology was discussed during an EFSA info session organised to discuss the methodology with the stakeholders. Also JRC has published several reports on assessment of mixtures, that advocate a new test strategy to define the relevant mixtures. EFSA takes pesticides as a concrete point of departure to develop strategies for dealing with mixtures. Such strategies, once developed, will then be generalised to other forms of mixtures. Central in this approach is the grouping of substances into so-called CAGs, i.e. cumulative assessment groups. Such CAGs are developed on the basis of similar target organs and/or mode of action (Rotter et al. 2019).

Several Member States (MS) also have issues reports and opinions on dealing with mixtures. For instance in the Netherlands, avoidance of cumulative exposures (of all environmental agents, not just substances) is one of the corner stones of modern environmental policy²⁹. In France, the new health law (currently under consideration) indicates that the identification of risks health should be done relying on the Exposome concept, integrating the effects of exposures to all non-genetic factors.

While there is a clear information need articulated from the side of policy makers, there is less insight in the possible action perspectives for policy makers and stakeholders in dealing with mixtures. Moreover, it is difficult to assess “value of information” for HBM data on mixtures: at what point would additional information on HBM and exposure to mixtures (based on HBM data, or the combined knowledge base) lead to other decisions and other/further policy actions? Should exposure to all substances in the mixtures be reduced, or the one with the highest impact on adverse health outcomes, the one with easy and safe alternatives/replacements, or the ones with the least costs to reduce, or should the cost-benefit ratio of each source/exposure route be taken into account. One can imagine that the cost-benefit ratio to reduce BPA exposure for babies, children, shop personnel, or in medical (emergency) equipment, may vary substantially.

Moreover, when mode of action (MoA) and adverse outcome pathways (AOP) are taken as point of departure to assess acceptability of the combined health impacts of exposure to mixtures, there may well be a need to compare across substances emerging from different types of applications, e.g. flame retardants, pesticides, plasticizers, and food additives/contaminants.

²⁹ Ministry of Infrastructure and the Environment (2014). Explicitly dealing with safety' (in Dutch) Bewust Omgaan met Veiligheid, Rode Draden; Een proeve van een lenM-breed afwegingskader veiligheid. 's Gravenhagen, Ministry of Infrastructure and the Environment.

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For HBM data on mixtures to be meaningful for policy development, it is necessary get further insight in and articulation of the expectations and primary policy needs already in the design phase of the research.

Based on, among other things, the above, the challenge from the Policy Board laid out for HBM4EU activities reads “We encourage the consortium to start addressing identification of chemical mixtures to which humans are exposed and develop concrete activities, across all three pillars, which would be carried out in the second half of the project. The pre-defined mixtures of substances having common mode of action could frame the initial perspective on this topic”.

16.1 Categorisation of Substances

Mixtures as a group fall into category C (Very little or no Human Biomonitoring data and/or information on toxicological/health effects or external exposure is available). While single chemicals, or even chemical family groups such as PCB's may warrant a category A or B classification, the essence of the mixture issue is the many unknowns about joint and cumulative exposure, combined mode of actions and overall adverse outcomes and health risks and impacts. Data coming available under category D and E would ultimately also fall under the Mixture umbrella.

16.2 Objectives / Policy-related questions

The overarching objective of the mixture activities in HBM4EU is to improve the efficacy of HBM to inform science, policy/regulatory actions and societal debate with respect to dealing with mixtures.

Some underlying questions include:

- ▶ What is the information need of regulatory bodies and stakeholders?
- ▶ What are common HBM mixture patterns in the European population and how do these unintentional mixtures vary across countries?
- ▶ Can we identify hotspots or risk groups with high mixture exposures?
- ▶ Which sources & pathways contribute most to HBM mixture values?
- ▶ What are the impacts of chemical mixtures on human health, and how can this inform risk assessment for mixtures, including EFSA's work on pesticides?
- ▶ What action perspectives are available to reduce mixture levels?

The more specific objectives are:

- ▶ Develop summary indicators to describe the exposure and body burdens of mixtures with an emphasis on defining priority mixtures and drivers of mixture toxicity
- ▶ Re-evaluate existing HBM mixture data to identify real-life exposure patterns to mixtures
- ▶ Collect new HBM mixture data in selected European countries
- ▶ Further develop and apply practical approaches to assess the potential health risks and impacts of mixtures by conducting case studies
- ▶ Inform policy makers, stakeholders and the public at large about mixture exposures, possible health risks and action perspectives

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16.3 Research Activities to be undertaken

Table 9: Listing of research activities to be carried out to answer the policy questions

Substance	Available knowledge related to policy question	Knowledge gaps / Activities needed to answer policy question
All	What is the information need of regulatory bodies and stakeholders?	In 2017 preparations started for develop exchange of information and establish cooperation amongst Horizon2020 funded projects on mixtures. To this end, two workshops have been organised, in which HBM4EU participated. This resulted in two publications (Bopp et al, 2018; Drakvik et al, 2020)
All	What are common HBM mixture patterns in the European population and how do these unintentional mixtures vary across countries?	In WP15, task 15.1 summary indicators are being developed to describe the exposure and body burdens of mixtures with an emphasis on defining priority mixtures and drivers of mixture toxicity. With these indicators we will re-evaluate existing HBM mixture data to identify real-life exposure patterns to mixtures and to obtain insight into the commonalities and differences across Europe.
All	Can we identify hotspots or risk groups with high mixture exposures?	In WP15, task 15.2, the SPECIMEn study is being conducted in five countries, with the aim to identify possible hotspots and risk groups.
All	Which sources & pathways contribute most to HBM mixture values?	In WP15, in concert with WP12, we are addressing source attribution to observed HBM mixture data
All	What are the impacts of chemical mixtures on human health, and how can this inform risk assessment for mixtures, including EFSA's work on pesticides?	In WP15, task 15.3, five case studies are being performed to obtain insight into the possibilities and limitations of existing methods for human health risk assessment of chemical mixtures
All	What action perspectives are available to reduce mixture levels?	In WP15, together with WP5, we will evaluate possible action perspectives and develop concrete recommendations for policy and further research

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17 Prioritised substance group: Mycotoxins

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17.1 Background information

Following a prioritisation strategy described in Deliverable 4.3, mycotoxins were selected as a prioritised substance group for 2019-2020. Given the wide variety of compounds comprised in the group of mycotoxins and following the view of the EU Policy Board, EFSA and DG SANTE, the focus was set on deoxynivalenol (DON) and fumonisin B1 (FB1).

Reviewers who provided comments: Marco Binaglia (EFSA, Italy), Rosa Lange (UBA, Germany), Greet Schoeters (Vito, Belgium), Argelia Castano (ISCI, Spain), Astrid Bulder, Marcel Mengelers (RIVM, The Netherlands), Hans-Mol (WUR, The Netherlands), Monica Olsen (National Food Agency, Sweden), Gabriele Sabbioni (AICT, Switzerland).

17.1.1 Hazardous properties

Mycotoxins are secondary fungal metabolites often found as natural contaminants in agricultural commodities all over the world and their occurrence pose a risk for human and animal health (Bennett and Klich, 2003; Wu et al., 2014). Generally, mycotoxins are chemically and thermally stable compounds, surviving storage and most production process (Koppen et al, 2010). Currently, the main human and animal health burdens of mycotoxin exposure are related to chronic toxicity, such as carcinogenic, teratogenic, immunotoxic, nephrotoxic, and endocrine disrupting effects. Chronic or even acute exposure to mycotoxins remains a daily fact, and therefore it is crucial that the mycotoxins' metabolism is unravelled so more knowledge on biomarkers in humans and animals is required.

The major foodborne mycotoxins of public health concern are the aflatoxins (e.g. aflatoxin B1, AFB1), fumonisins (e.g. fumonisin B1, FB1), trichothecene mycotoxins (e.g. deoxynivalenol, DON), and ochratoxin A (OTA) (Wu et al, 2014). These are produced primarily by fungi of the genera *Aspergillus*, *Fusarium*, and *Penicillium*, which commonly infect food crops. The International Agency for Research on Cancer (IARC) classified some mycotoxins from carcinogenic to humans (e.g. aflatoxin B1, group 1) to not classifiable regarding its carcinogenicity to humans (e.g. deoxynivalenol, group 3) (IARC 1993, 2002, 2012; Ostry et al, 2017). In the coming decades climate change is expected to impact fungal growth and agricultural practices (Paterson & Lima, 2011; Battilani et al, 2016; Sundheim et al, 2017) and, consequently, mycotoxins' concentrations and incidence in crops leading to an increase in human dietary exposure; (WHO, 2018; Assunção et al, 2018a).

DON and FB1 were prioritised in the 2nd round of substance prioritisation under HBM4EU and therefore, a more detailed review will be performed related to these mycotoxins.

Although there are structural alerts for DON as a suspected mutagen and carcinogen (Toolbox profiler Carcinogenicity by ISS) EFSA considers that DON is devoid of genotoxic potential (EFSA,

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2014). Accordingly, IARC considers that there is inadequate evidence in experimental animals for its carcinogenicity (group 3, IARC, 1993).

Its hepatotoxicity has been shown (Peng et al., 2017) although has not been consensual and thereby a systematic discussion of the hepatic toxicity of DON is needed. DON is suspected to be toxic for reproduction and it is able to cross the human placenta (Nielsen et al., 2011). In addition, its teratogenic potential has been shown in animals (Yu et al., 2017) and deserves to be further studied. DON (and other trichothecenes), is immunotoxic, acting as a potent inhibitor of protein synthesis and stimulating the pro-inflammatory response (Sundheim et al., 2017). EFSA CONTAM Panel established a group TDI of 1 µg/kg bw per day for the sum of DON and its acetylated and modified forms (3-Ac-DON, 15-Ac-DON and DON-3-glucoside) based on reduced body weight gain in mice.

In order to assess the acute human health risk, epidemiological data from mycotoxicoses were assessed and a group-ARfD of 8 µg/kg bw per eating occasion was calculated (EFSA, 2017).

FB1 is a suspected carcinogen according to the CLP classification and it is classified by IARC as possibly carcinogenic to humans (Group 2B, IARC, 2002). In vivo studies have shown that the repeated exposure to this toxin leads to liver and kidney toxicity (EFSA, 2018) and it is able to induce the formation of liver and kidney tumours (IARC, 2002). FB1 is not mutagenic in bacteria but it induces oxidative stress, being clastogenic to mammalian cells (EFSA, 2018). FB1 adverse effects are mainly mediated by the inhibition of ceramide synthases, which are key enzymes in sphingolipid metabolism. Based on the results of animal studies, JEFCA considered FB1 as a potential immunotoxic substance (WHO, 2011). It also causes developmental toxicity in several animal species (IARC, 2002). To derive HBGV for FB1, megalocytic hepatocytes in male mice were considered as the most appropriate outcome and a benchmark dose lower confidence limit 10% (BMDL10) of 165 µg/kg bw per day for FB1 was established (EFSA, 2018). The CONTAM Panel used the BMDL10 of 0.1 mg/kg bw per day and an uncertainty factor of 100 for intra and interspecies variability resulting in a TDI of 1.0 µg FB1/kg bw per day. Based on structural similarity and the limited data available indicating similar MoA and similar toxic potencies, the Panel decided that FB2, FB3 and FB4 should be included in a group TDI with FB1 (EFSA, 2018).

Recent surveys have highlighted the fact that humans are more frequently exposed to multiple than to single mycotoxins (Alvito et al, 2010; Grenier & Oswald, 2011; Solfrizzo et al., 2014; Alassane-Kpembi et al, 2016; Assunção et al, 2016), raising a concern about their potential combined effect on human health. The presence of DON, FB1 and other mycotoxins was reported in foods (Sirot et al, 2013; De Boevre et al, 2013; Garcia-Moraleja et al, 2015; Assunção et al, 2018; Martins et al, 2018), in biological samples from general population (Heyndrickx, 2015; Vidal et al, 2018; Brera et al, 2015; Escriva et al, 2017; Arce-Lopez, 2020) and in occupational settings (Fromme et al, 2016; Viegas et al, 2018, 2018a). Besides the regulated mycotoxins, an increasing number of studies are paying attention to mixtures involving the “emerging” ones (beauvericin, enniantins, Alternaria toxins, etc.) (Alassane-Kpembi et al, 2017; Gruber-Dorninger et al, 2017; Puntischer et al, 2018). Other authors also refer the possible interactions between environment and food contaminants, cadmium and deoxynivalenol, in different target organs (Thanh-Huong, L et al., 2018).

17.1.2 Exposure characteristics

Mycotoxins are commonly detected in cereal-based foods, cereals or fruit-based beverages, and several animal products (Bennett and Klich, 2003) and the general population is currently exposed by the oral route, via the ingestion of contaminated foods. Additional exposure routes include inhalation and dermal absorption, which can be particularly relevant for occupational exposure (Fromme et al, 2016; Viegas et al, 2015; Viegas et al, 2018).

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Results of the BIOMIN Mycotoxin Survey conducted from January to March 2018 indicate that deoxynivalenol (DON) and fumonisins (FUM) are the most common mycotoxins found in food commodities and feedstuffs (<https://www.biomin.net/en/biomin-mycotoxin-survey/>).

DON is the most prevalent *Fusarium* toxin in European grains and its occurrence is frequently reported in cereals and cereal-based products such as bread, pasta, or beer (Marin et al., 2013), thereby main exposure is by oral route. A total of 72,011 results of DON and its metabolites in food were obtained from 27 reporting countries and were related to samples collected between 2007 and 2014 (EFSA, 2017).

According to EFSA (2017), the estimated chronic dietary exposure was above the TDI of 1 µg/kg bw/day for infants, toddlers and other children regarding the mean exposure scenario, and for adolescents and adults regarding the high exposure scenario, thus indicating a potential health concern. The EFSA CONTAM Panel noted that the overall human dietary exposure to the sum of DON and its metabolites, 3-Ac-DON, 15-Ac-DON and DON-3-glucoside was mainly driven by DON (EFSA, 2017). DON and DON-3-glucoside were absorbed, distributed, metabolised and rapidly excreted through urine as shown recently by a human intervention study after exposure to DON and DON-3-glucoside (Vidal et al, 2018). The analysis of 24h urine samples revealed that DON-15-glucuronide was the most prominent urinary biomarker followed by free DON and DON-3-glucuronide. Based on this study, recently, and for the first time, a biokinetic model was developed to describe the renal excretion of DON and DON3G in humans. These models enable to determine the preferred (set of) urinary biomarker(s) (namely DON-15-glucuronide or total DON), the preferred urinary collection period (24 h), and to estimate the dietary exposure to these mycotoxins, by means of a reversed dosimetry factor (Mengelers et al, 2019).

Other studies have reported the detection of DON (total DON) in the urine of the general population in UK (Turner et al, 2010a), France (Turner et al, 2010b), Sweden (Turner et al, 2010), Italy (Solfrizzo et al, 2014), Croatia (Sarkanj et al, 2013), Austria (Warth et al, 2012), Belgium (Huybrechts et al, 2014), Germany (Gerding et al, 2014) and Portugal (Martins et al, 2019).

Females and males show different patterns of exposure levels, and human exposure to DON also shows some geographical differences (Chen et al, 2017; Vidal et al, 2018). Additional exposure by inhalation in occupational settings were also reported (Fromme et al, 2016; Viegas et al, 2018).

The occurrence of FB1–3 is well documented in maize and products thereof and the main exposure route is the oral route (EFSA, 2018). Animal studies indicate that FB1 is poorly absorbed from the gastrointestinal tract and rapidly cleared from the blood by the biliary route, and preferentially excreted with the faeces (EFSA, 2018). In Human Biomonitoring studies FB1 has been detected in urine of the general population in Sweden (Wallin et al, 2012), Austria (Warth et al, 2012), Belgium (Ediage et al, 2012), Germany (Gerding et al, 2014) and Portugal (Martins et al, 2019). Despite the low excretion rates for FB1 (0.93-2.6%) it has been proposed as biomarker of exposure. (Shephard et al. 1994; Dilkin et al. 2010; Gambacorta et al. 2013; Souto et al. 2017).

17.1.3 Policy relevance

In Europe, the European Commission (EU) has introduced comprehensive mycotoxin regulations for food to facilitate world trade and protect consumer's health (Eskola et al., 2018). The EU Regulation (EC) No 1881/2006 established the maximum permissible limits for aflatoxins (AFB1, sum of AFB1, AFB2, AFG1 and AFG2, AFM1), ochratoxin A (OTA), patulin (PAT), DON, zearalenone (ZEN), FBs (sum of FB1 and FB2), sum of T-2 and HT-2 toxins, and citrinine in specific food products (EC, 2006a and its amendments). This regulation also includes much lower regulatory limits for food for infants and young children due to their particular vulnerability and different consumption pattern. In addition to mycotoxin maximum levels, EU Regulation (EC) No

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401/2006 provides sampling plans according to nine different groups of food commodities taking into account the heterogeneous distribution of mycotoxins in agricultural commodities (EC, 2006b). The issue of effects resulting from exposure to multiple toxins (combined effects) and from different routes (aggregated exposure) had particularly concerned policy makers because combined effects can differ from individual effects of each chemical contaminant (Bouaziz et al, 2008). Government and industry regulations are usually based on individual mycotoxin toxicities and do not take into account the complex dynamics associated with interactions between co-occurring groups of mycotoxins (Assunção et al., 2016; Kienzler et al., 2016).

Farmers need to continuously assess the risk from mycotoxins to both crops and animals. These good practices together with harmonised international legislation on permitted maximum levels will ensure that highly contaminated cereals do not enter the food chain. From growers to retailers, all food business operators following the rules set by Codex Alimentarius Committee are able to ensure that food is safe in every home (Codex Committee on Contaminants in Food).

Concerning inhalation, the absence of exposure limits makes it difficult to interpret the exposure values and to determine acceptable values for occupational settings, in order to ensure workers' health (Viegas et al., 2018; Viegas et al., 2018a).

17.1.4 Technical aspects

Mycotoxin exposure assessment throughout biomonitoring studies based on the analysis of mycotoxin themselves, protein or DNA adducts, and/or major phase I or phase II metabolites (e.g. glucuronide conjugates), in human biological samples such as urine, serum and breast milk, have provided useful information over recent years. Fast advances in LC–MS technology have allowed multiple mycotoxins to be analysed simultaneously (Ediage et al, 2012; Warth et al, 2013; Solfrizzo et al, 2014; EFSA, 2017; Sarkanj et al., 2018).

Recent progress in biomarker research has allowed the determination of DON and its metabolites in urine, primarily as DON-glucuronides, by using single or multiple biomarker methods. DON-15-glucuronide, the sum of DON-glucuronides, or total DON (sum of free DON + DON-glucuronides after deconjugation) are considered suitable DON-biomarkers of exposure in urine. DON-3-glucoside, a modified form of DON, has a similar excretion profile as DON with DON-15-glucuronide being the most abundant metabolite (Vidal et al, 2018). To determine the urinary glucuronides, a preliminary approach was developed based on the enzymatic hydrolysis of deoxynivalenol-glucuronides, and subsequent determination of the “total DON” (sum of free and released mycotoxins by hydrolysis) (Solfrizzo et al, 2014; Turner et al, 2010). Afterwards, a direct method for quantification of glucuronides such as DON-3-glucuronide and DON-15-glucuronide was developed using in-house synthesised mycotoxin-standards (Ediage et al, 2012; Warth et al, 2013).

However, to not underestimate the total deoxynivalenol exposure in urine, the direct and indirect methodologies need to be compared since recent results revealed determination of total urinary deoxynivalenol does not convert all glucuronide forms to free deoxynivalenol (Vidal et al, 2020). These analytical developments permitted the scientific community to find strong correlations between the sum of urinary DON and its glucuronides (Vidal et al, 2018). Most of the reported analytical methods for DON biomarker analysis in urine were sensitive enough to differentiate exposure levels. However, commercial sources for DON glucuronide standards are scarce and no certified reference materials are available for urinary DON biomarkers (EFSA, 2017). New trends in high-resolution MS for untargeted metabolic profiling and metabolomics may unravel and identify novel metabolites, biotransformation products and/or modified DON forms (EFSA, 2017; Vidal et al, 2018; Sarkanj et al, 2018). Recently, DON-3-sulfate, a novel human metabolite and potential new biomarker of DON exposure was also reported in urine samples obtained from pregnant

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women in Croatia (Warth et al, 2016). Exposure to fumonisins can be assessed using urinary biomarkers. FB1–3 and hydrolysed form of FB1, HFB1, have been suggested as direct biomarkers of exposure by several authors (Shephard et al., 2007; Ediage et al, 2012; Heyndrickx et al., 2015). However, because of the poor urinary excretion of fumonisins and the consequent need for high sensitivity analytical procedures, the sample protocol requires an extensive clean-up and concentration step, based on SPE C18 cartridge or immunoaffinity purification. (EFSA, 2018).

17.1.5 Societal concern

It has been well recognised for many years that large economical losses occur worldwide owing to the mycotoxin contamination in agricultural products as recently summarised by Pitt and Miller (2016). Climate change is expected, in the upcoming decades, to impact fungal growth and agricultural practices and, consequently, to shift mycotoxins incidence, concentration and geographical spread.

The changing climate conditions will also lead to higher human and animal dietary exposure and, consequently, to increased human health risks (Wu and Mitchell, 2016). A recent report from WHO (2018) also refers to the effects that climate change could have on mycotoxins occurrence in Europe and their impact on human health. Moretti et al (2019) underlined that an extension of the aflatoxin contamination risk in maize in South and Central-Europe is highly likely in the next 30 years, due to favorable climatic conditions to the growth of *Aspergillus flavus*. Moreover, the mycotoxigenic *Fusarium* species profile on wheat in Europe is in continuous change in Northern, Central and Southern-Europe with, in particular, a worrisome growing contamination of *F. graminearum* in the Central and Northern Europe.

In this context, health effects resulting from exposure to multiple mycotoxins (combined effects) and from different routes (aggregated exposure) constitutes a rising concern, especially because health effects resulting from multiple mycotoxins exposure could lead to different output toxicity and carcinogenicity than exposure to single mycotoxins (Bouaziz et al., 2008). A multidisciplinary effort should be developed to perform the human health risk assessment of multiple mycotoxins present in food, considering that the information obtained from the risk assessment process will be used by risk managers to prioritise possible public health concerns and to develop risk management options towards disease prevention (Assunção et al, 2018).

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17.2 Categorisation of Substances

Table 17-1: Substances included in the mycotoxins group, DON and FB1, listed according to availability of toxicology and human biomarker data, in category C substances*

Category	Designation (Abbrev/ Acronym)	Systematic name (IUPAC name?)	CAS No.	Regulation
C	Deoxynivalenol (DON)	trichothec-9-en-8-one, 12, 13-epoxy-3,7,15- trihydroxy-, (3 α ,7 α)	51481-10-8	Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs Commission Regulation (EC) No 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs
C	Fumonisin B1 (FB1)	(2R)-2-[2- [(5R,6R,7S,9S,11R,16R,18S,19S)-19- amino-6-[(3R)-3,4- dicarboxybutanoyl]oxy-11,16,18- trihydroxy-5,9-dimethylcosan-7-yl]oxy- 2-oxoethyl]butanedioic acid	116355-83-0	Sum B1+B2 Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs Commission Regulation (EC) No 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs

**HBM scarcely exists, efforts to develop an analytical method to obtain relevant HBM results need to be done, hazardous properties of the substances are identified, yet greater knowledge on toxicological characteristics and effects on human health is needed, interpretation of HBM data is not possible, due to the lack of HBM guidance values.*

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17.3 Policy-related questions

The following questions are mandatory for deoxynivalenol (DON) and its acetylated and modified forms and fumonisin B1 (FB1). Data on other mycotoxins could be added, if possible.

1. Are there validated and harmonised analytical methods to assess the target mycotoxins exposure?
2. What is the current exposure levels of the European population to DON and FB1? Are there exposure data for other mycotoxins?
3. Does the exposure to mycotoxins differ among different population groups? Which are the main factors related with these differences (age, gender, settings, geographic localisation)?
4. Is there a time trend in human exposure to mycotoxins across Europe? Which are the identifiable factors associated with these trends (regulation related with food safety, climate change, others)?
5. Is the risk associated to human exposure to these mycotoxins characterised?
6. Are there exposure models and toxicokinetics data for mycotoxins and which are their limitations?
7. Is it possible to set a HBM guidance value for mycotoxins?
8. Which are the key-events that determine the long-term health effects from low-dose continuous exposure to the target mycotoxins?
9. Which are the most reliable and informative AOP-based effect biomarkers for prioritised mycotoxins?
10. Which research needs and gaps on target mycotoxins HBM?

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17.4 Research activities to be undertaken

Table 17-2: Listing of research activities to be carried out to answer the policy questions

Policy question	Substance	Available knowledge	Knowledge gaps (G) and activities needed (A)
1. Are there validated and harmonised analytical methods to assess the target mycotoxins exposure?	mycotoxins	<p>Analytical methods for DON and its glucuronides as well as FB1–4 are mainly based on LC-MS/MS.</p> <p>However, commercial sources for DON glucuronide standards are scarce and no certified reference materials are available for urinary DON biomarkers</p> <p>Only FB1–3 are available on the market as calibrant solutions, while FB4 can be purchased as purified powder. Except for HFB1, analytical standards for modified forms are not commercially available.</p>	<p>G: Current analytical methods, harmonised methods, reference materials, proficiency tests, expert laboratories</p> <p>A: 1. Identify across Europe the analytical capacity for determination of biomarkers of exposure, availability of reference materials and standards; best biomarkers, matrices and methods (Y3; WP9)</p> <p>2. Promote training and harmonisation on analysis at least of one selected compound: DON (total after deconjugation) including the organisation of an interlaboratorial comparison study (Y3, 4; WP2, WP9)</p> <p>3. Selection of expert laboratories to conduct the interlaboratorial study (Y3; WP9)</p> <p>4. Analysis by selected labs of samples from the aligned studies (Y3, 4; WP9)</p>

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Policy question	Substance	Available knowledge	Knowledge gaps (G) and activities needed (A)
2. What is the current exposure levels of the European population to DON and FB1? Are there exposure data for other mycotoxins?	Mycotoxins DON and FB1 are mandatory but HBM data on other mycotoxins are also welcome	Wide exposure to mycotoxins have been reported mainly through food commodities. Additional studies also report exposure by inhalation in occupational settings. DON (total DON) and FB1 were detected in the urine of the general population in United Kingdom, France, Sweden, Italy, Croatia, Austria, Belgium, Germany, Portugal, as well as in occupational settings (although in a lower extent).	G: Current data on mycotoxin exposure from EU countries for general population (different population groups) and, if possible, workers. A 1. Perform a literature search to identify studies developed in Europe that characterise exposure levels to DON and FB1 (Y3; WP10) 2. Invite HBM data owners to make their data available to perform exposure studies at European level (Y3; WP10) A3. Perform an analysis of the exposure levels at a European level (in the general population and/or in population subgroups) by combining HBM data on DON and FB1 obtained in the studies identified in 1 (Y3, WP10) 3. If possible integrate HBM data from the identified studies into IPChem (Y3,4; WP10) 4. Perform an analysis of HBM data from aligned studies on adult population European levels for prioritised mycotoxins (Y3,4; WP7, 8,10) 5. Perform an analysis of HBM data from other mycotoxins than the prioritised ones from literature search and aligned studies (Y3,4; WP7,8,10)
3. Does the exposure to mycotoxins differ among countries and different population groups? Which are the main factors related with these differences (age, gender, settings, geographic localisation)?	mycotoxins	Females and males show different excretion patterns, and human exposure to DON also shows some geographical differences. Occupational exposure revealed exposure associated with professional activity.	G: Current risk groups related to age, gender, occupational setting, location, in EU A: 1. Identify risk groups, including highly exposed, vulnerable and hotspots in Europe (Y3,4; WP10) 2. Perform statistical analyses based on the developed research protocols using collected HBM data from literature search and aligned studies, on geographical differences and exposure determinants related to mycotoxins (Y3; WP10).

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Policy question	Substance	Available knowledge	Knowledge gaps (G) and activities needed (A)
4. Is there a time trend in human exposure to mycotoxins across Europe? Which are the identifiable factors associated with these trends (regulation related with food safety, climate change, others)?	mycotoxins	<p>More than half of all worldwide agricultural samples contain DON and FUM (Biomimic Mycotoxin Survey).</p> <p>A total of 72,011 results of DON and its metabolites in food were obtained from 27 reporting countries and were related to samples collected between 2007 and 2014 (EFSA, 2017).</p>	<p>G: Analysis of trends on HBM mycotoxin exposure</p> <p>A: 1. Assess the occurrence of collected HBM data that will allow to proceed with a search on trends on HBM mycotoxin exposure.</p> <p>2. Identify possible temporal trends related to HBM mycotoxin exposure taking seasonal variation into account (Y4; WP10)</p> <p>3. Identify possible measures that could be associated with temporal trends (Y4; WP10)</p> <p>4. Identify possible reasons for the differences founded (Y4; WP10)</p>
5. Is the risk associated with human exposure to these mycotoxins characterised?	mycotoxins	<p>The estimated mean chronic dietary exposure for DON was above the group-TDI in infants, toddlers and other children, and at high exposure also in adolescents and adults, indicating a potential health concern.</p>	<p>G: Risk assessment (RA) and risk characterisation</p> <p>A: 1. Identify available estimates of human exposure via biomarkers (Y3,4; WP5)</p> <p>2. Collect toxicological data (Y3,4; WP5)</p> <p>3. Perform RA and risk characterisation of prioritised mycotoxins based on HBM data from literature search and aligned studies for adults (Y4; WP5)</p>
6. Are there exposure models and toxicokinetics data for mycotoxins and which are their limitations?	mycotoxins	<p>A biokinetic model was developed, for the first time, to describe the renal excretion of DON and DON3G in humans (Mengelers et al, 2019).</p> <p>Very few information is available on the toxicokinetics of FB1 in humans. Riley et al. (2012) determined the relation between fumonisins and their urinary excretion in humans. Only approximately 1% of the FB1 intake was excreted in the urine. Relating the urinary FB1 levels to the dietary FB1 intake in individuals is therefore difficult.</p>	<p>G: Exposure models and toxicokinetics in humans, especially for FB1.</p> <p>A: 1. Review of exposure models and toxicokinetics data for mycotoxins DON and FB1</p> <p>2. Explore the possibility of applying the previously developed toxicokinetic model for DON (Y3; WP12)</p> <p>3. Determine external exposure levels for DON from internal levels (based on HBM databases and available literature) through reverse dosimetry models (Y4; WP12)</p> <p>4. Bibliographic survey on biological half-lives for the prioritised substances</p>

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Policy question	Substance	Available knowledge	Knowledge gaps (G) and activities needed (A)
7. Is it possible to set a HBM guidance value for mycotoxins?	mycotoxins	<p>DON and its metabolite DON-3-glucoside were absorbed, distributed, metabolised and rapidly excreted through urine as shown recently by a 1st human intervention study after exposure to DON and DON-3-glucoside. This model could be used to set a HBM guidance value for DON for the general population.</p> <p>Animal studies indicate that FB1 is poorly absorbed from the gastrointestinal tract (less than 4% of the dose), rapidly cleared from the blood (with half-lives of less than 4 h) by the biliary route, and preferentially excreted with the faeces (usually more than 90% of the dose). No HBMGV available for biological samples. Due to the absence of kinetic studies on FB1 it is not likely that a HBM-GV can be derived in 2021.</p>	<p>G. Absence of HBMGV for prioritised mycotoxins</p> <p>A.1. Derivation of HBM HBGVs for the general population and, if possible, workers (Y4; WP5)</p>

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Policy question	Substance	Available knowledge	Knowledge gaps (G) and activities needed (A)
8. Which are the key events that determine the long-term health effects from low-dose continuous exposure to the target mycotoxins?	mycotoxins	<p>DON is considered as immunotoxic, reprotoxic and a probable endocrine disruptor. There is limited evidence on its potential genotoxicity and carcinogenicity. It is a potent inhibitor of protein synthesis and stimulates the pro-inflammatory response leading to oxidative stress. There's no good link between low-dose chronic exposure to DON and a human health effect.</p> <p>FB1 is a liver and kidney toxicant and it is immunotoxic. It is a probable carcinogen but there are data gaps on its mutagenicity. Its adverse effects are mainly mediated by the inhibition of ceramide synthases, which are key enzymes in sphingolipid metabolism. There are indications (based on animal studies and 1 study in humans) that chronic exposure to FB1 can be linked to a human health effect.</p>	<p>G: Several adverse health effects known (in animals) and mechanistic data available but AOP for DON and FB1 lacking</p> <p>A. 1. Identify for DON and FB1 the human health effect for which a AOP might be developed, e.g. immunotoxicity for DON and liver toxicity for FB1 (Y3; WP13).</p> <p>2. Disclose the key-events for the effects referred in 1. in order to contribute to AOPs development (Y3, Y4; WP13).</p>
9. Which are the most reliable and informative AOP-based effect based biomarkers for prioritized mycotoxins?	mycotoxins	Some biomarkers of early biological effects have been pointed for DON (e.g., pro-inflammatory cytokines) and FB1 (e.g., sphinganine-to-sphingosine ratio in blood) but further knowledge is needed	<p>G: Limited information on available biomarkers of effects</p> <p>A:1. Identify available targeted and untargeted biomarkers of effect for the selected mycotoxins (Y3, Y4; WP14).</p>

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Policy question	Substance	Available knowledge	Knowledge gaps (G) and activities needed (A)
10. Which research needs and gaps on Human Biomonitoring activities related to target mycotoxins?	mycotoxins	Important challenges that should be faced in mycotoxin Human Biomonitoring domain report the need of update mycotoxins HBM data to assess and characterise the risk of European human exposure to mycotoxins, increase knowledge of mycotoxin metabolism and toxicokinetics, derivation of HBGV to perform a better RA and risk characterisation, the need for validated methodologies and reference materials and new methodologies for treating samples.	<p>G. Lacks of knowledge on mycotoxins Human Biomonitoring</p> <p>A. 1 Validated and harmonised analytical mycotoxins methods, standards and reference materials</p> <p>2. Updated HBM exposure data, to perform a more accurate RA and risk characterisation</p> <p>3. HBM GV and reference values</p> <p>4. Toxicokinetic models</p> <p>5. AOPs & effect biomarkers</p>

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18 Prioritised substance group: PAHs and air pollutants

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18.1 Background Information

18.1.1 Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants generated primarily during the incomplete combustion of organic materials (e.g. coal, oil, petrol, and wood). Emissions from anthropogenic activities predominate (automobile emissions and cigarette smoke); nevertheless, some PAHs in the environment originate from natural sources (e.g. open burning, natural losses or seepage of petroleum or coal deposits, and volcanic activities).

Particulate Matter is generally categorised on the basis of the size of the particles that reflects their aerodynamic diameter (e.g. PM_{2.5} refers to particles with an aerodynamic diameter of less than 2.5µm). PM is made up of a wide range of components and are formed from a variety of sources and processes. Ambient air levels of PM comprise primary particles emitted directly into the atmosphere from combustion sources and secondary particles formed by chemical reactions in the air. Ambient air PM are released from both anthropogenic and natural sources (such as sea spray, Saharan dust or volcanos). The most common anthropogenic sources are stationary fuel combustion and transport. Road transport gives rise to primary particles from engine emissions, as well as various non-exhaust emissions such as tire and brake wear. Secondary PM is formed from emissions of ammonia, sulphur dioxide and oxides of nitrogen as well as from emissions of organic compounds from both combustion sources and vegetation.

Ozone is not emitted directly from anthropogenic sources; it is formed by photochemical reactions resulting from the interaction of sunlight on nitrogen dioxide (NO₂) and VOCs, typically emitted from transportation sources. Formation can take place over several hours or days and may have arisen from emissions many hundreds, or even thousands of kilometers away. Ozone is a secondary pollutant, which often impacts rural areas far from the original emission site as a result of long-range transport.

Sulphur dioxide (SO₂) emissions are dominated by combustion of fuels containing sulphur, such as coal and heavy oils by power stations and refineries. In some EU countries, coal for domestic use is a significant source. Carbon monoxide (CO) is mainly formed from incomplete combustion of carbon containing fuels. The most significant source is road transport, followed by residential and industrial combustion.

Nitrogen Dioxide (NO₂) is one of a group of gases called nitrogen oxides (NO_x). While all of these gases are harmful to human health and the environment, NO₂ is of the greatest concern. NO₂ primarily gets in the air from the burning of fuel related to transport emissions, mainly from diesel vehicles (including cars, trucks and buses) and power plants.

Finally, benzene is an air toxic emitted from gasoline service stations, gasoline (mainly) motor vehicle exhaust and fuel evaporation, the burning of coal and oil, and to a lesser extend to various other combustion sources.

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18.1.2 Hazardous properties

Many PAHs are known or suspected carcinogenic and mutagenic compounds (e.g., benzo(a)pyrene, dibenzo(a,h) anthracene, etc.). They are included in the candidate list under article 59 of REACH which contains a number of complex substances derived from petroleum and coal such as: coal tar pitch, high temperature (CTPHT) – EC 266-028-2; anthracene oil EC 292-602-7 and other anthracene related fractions. The reasons for inclusion are the Persistent Bioaccumulative Toxic (PBT), very Persistent very Bioaccumulative (vPvB) and carcinogenic properties of the PAHs which are present as constituents in these UVCB substances (substances of Unknown or Variable composition, Complex reaction products or Biological materials, ECHA)

Currently eight PAH congeners (Benzo[a]pyrene (BaP), benzo[e]pyrene (BeP), benzo[a]anthracene (BaA), chrysene (CHR), benzo[b]fluoranthene, (BbF), benzo[j]fluoranthene (BjF), benzo[k]fluoranthene (BkF), dibenzo[a,h]anthracene (DBAhA)) are classified as known carcinogens in Annex VI of Regulation (EC) 1272/2008 (Classification Labelling and Packaging, CLP regulation). These are legally classified carcinogens of Category 1B acc. to the CLP regulation.

Benzo[a]pyrene (BaP) and chrysene (CHR) are also legally classified mutagens (CLP Cat. 1B; CHR: CLP Cat. 2). In addition, BaP is a classified reprotoxicant (CLP: Cat. 1B). Lack of 'CMR (Carcinogenic Mutagenic Reprotoxic)' classification¹ for the other PAH congeners may rather be attributed to the comparatively limited database available for these compounds. There are indications that the carcinogenic potency of some further PAH congeners, e.g. some of the dibenzopyrenes, may even be considerably higher than that of the lead compound BaP.

The mechanism of toxicity is considered to be interference with the function of cellular membranes as well as with enzyme systems which are associated with the membrane. It has been proven that PAHs can cause carcinogenic and mutagenic effects and are potent immune-suppressants. Effects have been documented on immune system development, humoral immunity and on host resistance [1-2]. PAH-induced carcinogenesis can result when a PAH-DNA adduct forms at a site critical to the regulation of cell differentiation or growth. A mutation occurs during cell replication if the aberration remains unrepaired. Cells affected most significantly by acute PAH exposure appear to be those with rapid replicative turnover, such as those in bone marrow, skin, and lung tissue. Tissues with slower turnover rates, such as liver tissue, are less susceptible. Target organs identified in animal studies with some of the PAHs were the skin, the liver, the hemolymphatic and the respiratory system [3-5]. Many PAHs are aryl hydrocarbon receptor (AhR) ligands and several recent studies have suggested that PAHs or their metabolites may activate estrogen receptors (ER). Activation of ER signaling in endocrine cancer prone tissues, such as breast epithelium, might thus further contribute to their known carcinogenicity [6]. PAHs have been shown to exert endocrine and developmental toxicity in experimental animals, including decreased weight of reproductive organs, damage to growing ovarian follicles, decreased fertility, embryonic damage and lethality or developmental defects of testis and spermatogenesis in males [7-9].

PAHs can be formed both during biological processes and as products of incomplete combustion from either natural combustion sources (forest and brush fires) or man-made combustion sources (automobile emissions and cigarette smoke). Thus, PAHs are commonly detected in air, soil, and water. Therefore, PAHs are considered ubiquitous in the environment [10, 11]. PAHs are highly lipid soluble and thus readily absorbed from the gastrointestinal tract of mammals.

They are absorbed through ingestion, inhalation, and dermal contact, according to animal study data. The percentage absorbed varies in these studies for several reasons, including the vehicle (transport medium) in which the PAHs are found [12]. In general, PAHs not bound to particulate matter may be absorbed in the lungs better than the same dose found on the surface of airborne particulate matter [13, 14]. They are rapidly distributed in a wide variety of tissues with a marked

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tendency for localisation in body fat. Metabolism of PAHs occurs via the cytochrome P450-mediated mixed function oxidase system with oxidation or hydroxylation as the first step. Because of their lipophilic nature, PAHs can accumulate in breast milk and adipose tissue. However, biliary and urinary excretion of PAHs is relatively efficient because of the wide distribution of enzymes that transform PAHs into polar metabolites.

PAHs are predominantly metabolized in the liver, via CYP enzymes (enzymes in the P-450 mixed-function oxidase system) [15-17].

In addition to the liver and kidneys, metabolism of PAHs occurs in the adrenal glands, testes, thyroid, lungs, skin, sebaceous glands, and small intestines [18].

PAHs are transformed initially to epoxides, which are converted to dihydrodiol derivatives and phenols. Glucuronide and sulfate conjugates of these metabolites are excreted in the bile and urine. Glutathione conjugates are further metabolised to mercapturic acids in the kidney and are excreted in the urine.

The hydroxylated metabolites of the PAHs are excreted in human urine both as free hydroxylated metabolites and as hydroxylated metabolites conjugated to glucuronic acid and sulfate [19]. A commonly measured urinary metabolite is 1-hydroxypyrene [20-22].

Metabolism is a prerequisite for hepatobiliary excretion and elimination through the feces, regardless of route of entry. Excretion half-lives in feces and urine have been reported in animal studies as 22 hours and 28 hours, respectively [21].

Pyrene is commonly found in PAH mixtures, and its urinary metabolite, 1-hydroxypyrene, has been used as an indicator of exposure to PAH chemicals [19-22].

Exposure to PAHs is almost always to mixtures that pose a challenge in developing conclusions [23]. Several epidemiologic studies have shown increased cancer mortality in workers exposed to PAHs.

Carbon Monoxide is a colourless, odourless, tasteless gas that is slightly lighter than air. Natural background levels of CO fall in the range of 10-200 ppb. Levels in urban areas are highly variable, depending upon weather conditions and traffic density. 8-hour mean values are generally less than 10 ppm (12 mgm⁻³) but have been known to be as high as 500 ppm (600 mgm⁻³). The European limit value for the maximum daily 8-hour mean concentrations of CO is set to 10 mg/m³ [24]. Based on the available measurements, it can be concluded that in EU the CO ambient concentrations above the limit value is very localised and infrequent, and is limited to a very few areas near traffic and industry. The only concentration above the limit value was registered at an urban industrial station in the former Yugoslav Republic of Macedonia [25].

CO is an intermediate product through which all carbon species must pass when combusted in oxygen (O₂). In the presence of an adequate supply of O₂ most CO produced during combustion is immediately oxidised to carbon dioxide (CO₂). However, this is not the case in spark ignition engines, especially under idling and deceleration conditions.

Thus, the major source of atmospheric CO is the spark ignition combustion engine. Smaller contributions come from processes involving the combustion of organic matter, for example in power stations and waste incineration.

The main health effects related to exposure to CO are: headaches, dizziness, slows mental processes, and at high levels can lead to death. CO prevents the normal transport of oxygen by the blood. This can lead to a significant reduction in the supply of oxygen to the heart, particularly in people suffering from heart disease.

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SO₂ is a colourless gas. It reacts on the surface of a variety of airborne solid particles, is soluble in water and can be oxidised within airborne water droplets.

Annual mean concentrations in most major UK cities are now well below 35 ppb (100 µg/m³) with typical mean values in the range of 5-20 ppb (15-50 µg/m³). Hourly peak values can be 400-750 ppb (1000-2000 µg/m³) on infrequent occasions. Natural background levels are about 2 ppb (5 µg/m³). The European air quality standards for SO₂ are defined by the Ambient Air Quality Directive [26] for SO₂. The limit value for 24-hour average SO₂ concentration is set at 125 µg/m³ and can be exceeded on up to 3 days per year. The 1-hour limit value threshold of 350 µg/m³ can be exceeded on up to 24 hours per year. EU countries were obliged to meet both public health protection limits by 2005. There is also an 'alert' threshold value of 500 µg/m³. When this alert threshold is exceeded over three consecutive hours, authorities have to implement action plans to lower the high levels of SO₂. SO₂ concentrations are generally well below the limit values for the protection of human health. Only one industrial station in Bulgaria, out of some 1 350 stations measuring SO₂ in 34 European countries (EU-28, Albania, FYROM, Iceland, Montenegro, Norway and Serbia), registered concentrations of SO₂ above this limit value.

The most important sources of SO₂ are fossil fuel combustion, smelting, manufacture of sulphuric acid, conversion of wood pulp to paper, incineration of refuse and production of elemental sulphur. Coal burning is the single largest man-made source of SO₂ accounting for about 50% of annual global emissions, with oil burning accounting for a further 25-30%.

Even moderate concentrations may result in constriction of the lung airways. This effect is particularly likely to occur in people suffering from asthma and chronic lung disease. Tightness in the chest and coughing occur at high levels, and lung function of asthmatics may be impaired to the extent that medical help is required. Sulphur dioxide pollution is considered more harmful when particulate and other pollution concentrations are high.

NO_x is a collective term used to refer to two species of oxides of nitrogen: nitric oxide (NO) and nitrogen dioxide (NO₂). Annual mean concentrations of NO₂ in urban areas are generally in the range 10-45 ppb (20-90 µg/m³). Levels vary significantly throughout the day, with peaks generally occurring twice daily as a consequence of "rush hour" traffic. Maximum daily and one hourly means can be as high as 200 ppb (400 µg/m³) and 600 ppb (1200 µg/m³) respectively. The Ambient Air Quality directive [26] sets short-term (1-hour) and long-term (annual mean) limit values for the protection of human health. The limit value for the annual mean NO₂ concentration is set at 40 µg/m³. The 1-hour limit value threshold of 200 µg/m³ can be exceeded on up to 18 days per year (corresponding to the 99.8 percentile of hourly concentrations in one year) before the limit value is breached.

In 17 (Austria, Belgium, the Czech Republic, Denmark, Finland, France, Germany, Greece, Italy, Latvia, the Netherlands, Poland, Portugal, Slovakia, Spain, Sweden and the United Kingdom) of the 28 EU Member States recorded concentrations above the annual limit value at one or more stations. Concentrations above 55 µg/m³ were also measured at one urban background station in Serbia and another in the United Kingdom. These findings demonstrate that NO₂ concentrations still need to be substantially reduced in large areas of Europe (focusing on traffic and urban locations) for the annual limit value to be met. The hourly limit value threshold for NO₂ is less stringent.

Concentrations above this limit value were observed in 0.5 % of all the reporting stations, mostly at urban traffic stations except for two (urban and suburban) background stations. They were observed in seven (Spain (five stations), Germany (three) and France, Hungary, Italy, Portugal and the United Kingdom (one station each)) Member States.

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Globally, quantities of nitrogen oxides produced naturally (by bacterial and volcanic action and lightning) far outweigh anthropogenic (man-made) emissions. Anthropogenic emissions are mainly due to fossil fuel combustion from both stationary sources, i.e. power generation (21%), and mobile sources, i.e. transport (44%). Other atmospheric contributions come from non-combustion processes, for example nitric acid manufacture, welding processes and the use of explosives.

The main health effects associated to exposure to NO_x are: shortness of breath or coughing and enhanced risk of respiratory disease. NO₂ is associated with several respiratory adverse effects on human health. At high levels NO₂ causes inflammation of the airways. Long term exposure may affect lung function and respiratory symptoms. NO₂ also enhances the response to allergens in sensitive individuals. Nitrogen dioxide can irritate the lungs and lower resistance to respiratory infections such as influenza. Continued or frequent exposure to concentrations that are typically much higher than those normally found in the ambient air may cause increased incidence of acute respiratory illness in children.

O₃ is the tri-atomic form of molecular oxygen. It is a strong oxidising agent, and hence highly reactive. Background levels of O₃ in Europe are usually less than 15 ppb but can be as 100 ppb during summer time photochemical smog episodes. In the UK ozone occurs in higher concentrations during summer than winter, in the south rather than the north and in rural rather than urban areas. According to the Ambient Air Quality Directive [26], a maximum daily 8-hour mean threshold of 120 µg/m³ has been established. The target applied by EU Member States (starting from January 1, 2010), is that the threshold should not be exceeded at a monitoring station more than 25 day per year (corresponding to the 93.2 percentile), determined as a 3-year average starting from 2010. The long-term objective is no exceedance of the threshold level at all. For public health protection, there are also two other types of thresholds: 'public information' (180 µg/m³) and 'alert' thresholds (240 µg/m³). When the public information threshold is breached, the authorities in that country are obliged to notify their citizens, using a public information notice. When the alert threshold is exceeded for three consecutive hours, a short-term action plan has to be drawn up, in accordance with the specific provisions established in the Ambient Air Quality Directive [26].

Since the formation of O₃ requires sunlight, O₃ levels increase as one moves from the northern to the southern parts of Europe, with the highest levels observed in some Mediterranean countries. The O₃ typical concentrations in 16 EU countries (Austria, Bulgaria, Croatia, Cyprus, the Czech Republic, France, Germany, Greece, Hungary, Italy, Luxembourg, Malta, Poland, Slovakia, Slovenia and Spain) of the EU-28 [25] were above the O₃ target value more than 25 times.

Most O₃ in the troposphere (lower atmosphere) is formed indirectly by the action of sunlight on nitrogen dioxide - there are no direct emissions of O₃ to the atmosphere. About 10 - 15% of tropospheric O₃ is transported from the stratosphere where it is formed by the action of ultraviolet (UV) radiation on O₂. In addition to O₃, photochemical reactions involving sunlight produce a number of oxidants including peroxyacetyl nitrate (PAN), nitric acid and hydrogen peroxide, as well as secondary aldehydes, formic acid, fine particulates and an array of short lived radicals. As a result of the various reactions that take place, O₃ tends to build up downwind of urban centres where most of NO_x is emitted from vehicles.

Exposure to high levels of O₃ may result in irritation to eyes and nose. Very high levels can damage airways leading to inflammatory responses. Ozone reduces lung function and increases incidence of respiratory symptoms, respiratory hospital admissions and mortality. Ground level ozone can also cause damage to many plant species leading to loss of yield and quality of crops, damage to forests and impacts on biodiversity.

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Particulate matter is a complex mixture of organic and inorganic substances, present in the atmosphere as both liquids and solids. Coarse particulates can be regarded as those with an aerodynamic diameter greater than 2.5 μm (micrometres), and fine particles less than 2.5 μm . Coarse particles usually contain earth crustal materials and fugitive dust from roads and industries. Fine particles contain the secondarily formed aerosols, combustion particles and re-condensed organic and metallic vapours. The acid component of particulate matter generally occurs as fine particles. A further distinction that can be made is to classify particulates as either primary or secondary, according to their origin. Primary particulates are those emitted directly to the atmosphere while secondary particulates are those formed by reactions involving other pollutants. In the urban context, most secondary particulate matter occurs as sulphates and nitrates formed in reactions involving SO_2 and NO_x .

Reported concentrations vary according to the sampling technique. In urban areas typical annual mean values are 10 - 40 $\mu\text{g}/\text{m}^3$ (gravimetric sampling) although short-lived pollution episodes such as Bonfire night can cause particulate concentrations to rise to several hundred $\mu\text{g}/\text{m}^3$. Background levels in rural areas range from 0-10 $\mu\text{g}/\text{m}^3$. The Ambient Air Quality Directive [26] sets limit values for long-term (annual) $\text{PM}_{2.5}$ concentrations. The deadline for meeting the target value for $\text{PM}_{2.5}$ (25 $\mu\text{g}/\text{m}^3$) was 1 January 2010, and the deadline for meeting the limit value (25 $\mu\text{g}/\text{m}^3$) and the exposure concentration obligation for $\text{PM}_{2.5}$ (20 $\mu\text{g}/\text{m}^3$) was 2015. The typical concentrations of $\text{PM}_{2.5}$ in EU is above the EU limit value in large parts of Europe according to the data of the European air-quality database ([25]. Nevertheless, there were stations with $\text{PM}_{2.5}$ concentrations higher than the target value (annual mean, which has been the limit value for $\text{PM}_{2.5}$ from 2015 on) in four Member States of EU. These concentrations were observed in Bulgaria (ranged from 25 to 30 $\mu\text{g}/\text{m}^3$), Czech Republic, Italy and Poland, as well as in one station in the former Yugoslav Republic of Macedonia [25]. The $\text{PM}_{2.5}$ concentrations in these countries ranged from 25 to 30 $\mu\text{g}/\text{m}^3$.

Particulate matter is emitted from a wide range of sources, the most significant primary sources being road transport (20%), homes (20%), construction, mining and quarrying (13%), industrial combustion plants and processes (10%) and public power generation (10%). Natural sources are less important; these include volcanoes and dust storms. Particulate matter can also be formed by the transformation of gaseous emissions such as oxides of sulphur and nitrogen and VOCs.

Both short-term and longterm exposure to ambient levels of PM are consistently associated with respiratory and cardiovascular illness and mortality as well as other adverse health effects. It is not currently possible to discern a threshold concentration below which there are no effects on public health. Fine particles are deposited in the lowest part of the human respiratory tract, where they can cause inflammation and a worsening of the condition of people with heart and lung diseases. In addition, they may carry surface-absorbed carcinogenic compounds into the lungs.

Benzene is a colourless, clear liquid compound. It is fairly stable but highly volatile, i.e. it readily evaporates. Ambient concentrations of benzene are typically between 1 - 50 ppb. Levels close to major emission sources can be as high as several hundred ppb.

The urban background mean concentration of benzene is 1 to 2 ppb (3 to 6 $\mu\text{g}/\text{m}^3$); rural areas average 0.5 to 1 ppb (1.5 to 3 $\mu\text{g}/\text{m}^3$). Mean annual concentration can be 5 ppb (15 $\mu\text{g}/\text{m}^3$) on urban roadsides. The limit value for benzene is set as an annual mean, given that C_6H_6 is a carcinogen with long-term effects. The target value for benzene is set at 5 $\mu\text{g}/\text{m}^3$. Nevertheless, C_6H_6 is measured at a relatively small number of stations in EU. The concentrations above the limit value is limited to a few local areas with higher concentrations (2 $\mu\text{g}/\text{m}^3$) which are often close to traffic or industrial sources. No exceedances of the limit value were observed [25].

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About 80% of man-made emissions come from petrol-fueled vehicles. This results from both the benzene content of the fuel and partial combustion of the petrol. A further 5% comes from the handling, distribution and storage of petrol and approximately 1% comes from oil refining. Emissions also come from benzene-producing and handling industries, the burning of wood and other organic material, and the use of benzene as a laboratory reagent.

Human exposure to benzene has been associated with a range of acute and long-term adverse health effects and diseases, including cancer and aplastic anemia. Benzene is a recognised human carcinogen that interacts with the genetic material and, as such, no absolutely safe level can be specified in ambient air. Studies in workers exposed to high levels have shown an excessive risk of leukemia. Exposure can occur occupationally and domestically as a result of the ubiquitous use of benzene-containing petroleum products, including motor fuels and solvents. Active and passive exposure to tobacco smoke is also a significant source of exposure. Benzene is highly volatile, and exposure occurs mostly through inhalation.

VOCs comprise a very wide range of individual substances, including hydrocarbons, halocarbons and oxygenates. All are organic compounds and of sufficient volatility to exist as vapour in the atmosphere. Methane is an important component of VOCs, its environmental impact principally related to its contribution to global warming and to the production of ozone in the troposphere. Regional effects derive from non-methane VOCs (NMVOCs), such as benzene and toluene.

Most measurements of total VOCs are in terms of their carbon content, without analysis as individual compounds. The major contributor to VOCs is normally methane with a local background concentration of 1.6 ppm. Whilst most other individual compounds (e.g. benzene) are present in urban air at concentrations of a few ppb, or less, total NMVOCs will amount to several hundred ppb concentrations.

Hydrocarbons are emitted from petrol evaporation and incomplete combustion, and from leakage of natural gas from distribution systems. Oxygenates arise in vehicle exhausts and via atmospheric chemical reactions. Evaporation of solvents, used in paints or industrial degreasing processes, cause a release of hydrocarbons, oxygenates and halocarbons to the atmosphere.

BaP is a potent carcinogen. The target value for BaP for the protection of human health is set at 1 ng/m³ [27] as an annual mean. Ambient air concentrations of BaP are high across large parts of Europe, mostly as a result of emissions from domestic combustion of coal and wood. More than a third of the BaP measurement stations in Europe measured annual concentrations above 1.0 ng/m³. Values above 1.0 ng/m³ were measured mainly at urban and suburban stations. These values above 1.0 ng/m³ (1.0 to 1.5 ng/m³) are most predominant in central and eastern Europe (Austria, Bulgaria, Croatia, the Czech Republic, Hungary, Italy, Lithuania, and Poland), although they are also found in Finland, Germany, Ireland and the United Kingdom. The average concentration measured at Polish stations is 4.8 times as high as the target value (ranging from 0.8 to 10 ng/m³). Possible chronic health effects include cancer, central nervous system disorders, liver and kidney damage, reproductive disorders, and birth defects.

18.1.3 Exposure characteristics

Combustion sources are thought to account for over 90% of the environmental concentrations of PAHs. Major anthropogenic sources of PAHs include residential heating, coal gasification and liquefying plants, carbon black, coal-tar pitch and asphalt production, coke and aluminum production, catalytic cracking towers and related activities in petroleum refineries as well as motor vehicle exhaust. In addition, there could be some other, mainly local/target group exposure from very defined uses of products emitting PAHs. Synthetic turf, made with an infill of rubber crumb from used tires or virgin rubber also contains PAHs.

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The following three types: *pyrogenic*, *petrogenic*, and *biological* are the major PAH sources to the environment. In pyrolysis processes pyrogenic PAHs are formed whenever organic substances are exposed to high temperatures under low oxygen or no oxygen conditions. The destructive distillation of coal into coke and coal tar, or the thermal cracking of petroleum residuals into lighter hydrocarbons are pyrolytic processes that occur intentionally. Meanwhile, other unintentionally processes occur during the incomplete combustion of motor fuels in cars and trucks, the incomplete combustion of wood in forest fires and fireplaces, and the incomplete combustion of fuel oils in heating systems.

The temperatures at which the pyrogenic processes occur are ranging from about (350 °C to more than 1200 °C). Pyrogenic PAHs are generally found in greater concentrations in urban areas and in locations close to major sources of PAHs.

PAHs formed during crude oil maturation and similar processes are called petrogenic. Such petrogenic PAHs are common due to the widespread transportation, storage, and use of crude oil and crude oil products. Some of the major sources of petrogenic PAHs include oceanic and freshwater oil spills, underground and above ground storage tank leaks, and the accumulation of vast numbers of small releases of gasoline, motor oil, and related substances associated with transportation. It is well-known that PAHs can be formed during the incomplete combustion of organic substances. PAHs are also found in petroleum products.

On the other hand, it is not well-known that PAHs can be produced biologically. For example, they can be synthesised by certain plants and bacteria or formed during the degradation of vegetative matter.

PAHs are also found in a multitude of consumer articles and mixtures. Although they are not produced intentionally for this purpose, they are present in these products due to the use of plasticisers (e.g. extender oils) or carbon black (soot) in the manufacture of rubber or other elastomers.

The atmosphere is the most important means of PAH dispersal, it receives the bulk of the PAH environmental load resulting in PAHs being ubiquitous in the environment.

Once released to the atmosphere, PAHs are found in two separate phases, a vapor phase and a solid phase in which the PAHs are sorbed onto particulate matter [28-30]. Hydrophobic organic chemicals with low vapor pressures, such as PAHs, are sorbed to atmospheric particulates more readily than chemicals with higher vapor pressures. The variability in vapor pressures of different PAH compounds cause the individual PAHs to distribute in different concentrations in the vapor [8] and other sorbed phases [31]. Low-molecular-weight PAHs (two and three rings) occur in the atmosphere predominantly in the vapour phase, whereas multi-ringed PAHs (five rings or more) are largely bound to particles. Intermediate-molecular-weight PAHs (four rings) are partitioned between the vapour and particulate phases, depending on the atmospheric temperature [32].

The removal of PAHs from the atmosphere by dry and wet deposition processes are strongly influenced by their gas/particle partitioning. Atmospheric deposition is a major source for PAHs in soil.

Background levels of some representative PAHs in the air are reported to be 0.02-1.2 ng/m³ in rural areas and 0.15-19.3 ng/m³ in urban areas. Background levels of PAHs in drinking water range from 4 to 24 ng/L.

Humans are exposed to PAH through several routes, namely inhalation of air and re-suspended soil and dust, consumption of food and water, and dermal contact with soil and dust [33]. All these sources are relevant to global human exposure

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There is no sufficient evidence that exposure to PAHs has declined during the last ten years in Europe. In terms of spatial differentiation, exposure to PAHs is expected to be higher in areas with intense traffic and industrial activity.

Personal lifestyle factors, such as smoking and the use of indoor biomass combustion for heating and cooking, are also important determinants of exposure.

Since certain PAHs are considered carcinogens, there is no threshold under which exposure is safe. Thus, there are no BE values for PAHs. The maximum levels of benzo(a)pyrene and the sum of benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene and chrysene are regulated in food stuff according to Commission Regulation (EU) No 835/2011 [34]. In addition, Entry 50 of Annex XVII of the REACH regulation also stipulates limits for PAH containing extender oils in car tyres [35]. According to the regulation the extender oils shall not be placed on the market, or used for the production of tyres or parts of tyres if they contain more than 1 mg/kg (0,0001 % by weight) BaP and more than 10 mg/kg (0,001 % by weight) of the sum of all listed PAHs.

In the Provisional Guidance for Quantitative Risk Assessment of Polycyclic Aromatic Hydrocarbons [36], United States EPA recommends using toxicity equivalency factors (TEFs) to convert concentrations of 19 carcinogenic PAHs (cPAHs) to an equivalent concentration of benzo(a)pyrene (B[a]P). In this scheme the TEF for B[a]P is set equal to one.

Urinary levels of PAHs and their respective metabolites are associated with proximity to combustion sources such as municipal solid waste incinerators [37]. Levels of 1-hydroxypyrene (1-OHP) (a major urinary PAH metabolite) were found to be higher for smokers (0.14 µg/g creatinine) than for non-smokers (0.08 µg/g creatinine) in the study by Lafontaine et al. [38], but not in the study by Leroyer et al. [39].

Proximity to industrial sites hot-spots in Germany was found to significantly affect PAH exposure levels with the mean urinary 1-OH-P level of 0.31 µg/g creatinine in the children living close to the hot spots, compared to 0.15 µg/g creatinine compared to children living far from hot spots [40]. In all cases, the 1-OH-P levels were lower than the reference value (RV) of 0.5 µg/L [41].

Studies in the Czech Republic [42, 43] found that levels of B[a]P-like DNA adducts were similar in the Ostrava and Prague regions, although B[a]P levels in the Ostrava region were more than eight times higher. This was attributed to the more efficient DNA repair capacity in the continuously highly exposed population. The nonlinear association between exposure levels and the formation of DNA-adducts, or the occurrence of oxidative stress, highlights the need to use advanced multi-omics approaches that can help to explain the observed pattern and reveal the mechanisms of interaction between environmental toxicants and human systems, which are modified by genetic make-up and other intrinsic factors.

Exposure to PAHs is affected by proximity to intense combustion sources, such as heavily trafficked roads, municipal waste incinerators and industrial sites. An additional source of PAHs is combustion of solid fuel for space heating. In this regard, special attention ought to be paid to the use of biomass in large urban and metropolitan areas, which, if not controlled, may contribute substantially to the overall PAH exposure of the urban population. Biomass combustion for heating is expected to contribute to indoor exposure as well.

For the rest of air pollutants, the only relevant route of exposure is inhalation. In practice, people are exposed to various levels of air pollutants during their daily activity, depending on a) the levels of these pollutants in the various microenvironments and b) the inhalation rate which is related to age, gender and the respective activity performed in the microenvironment. The concentration levels of the pollutants are clearly linked to the proximity to major sources, e.g. proximity to heavily trafficked roads results in increased levels of traffic related pollutants such as PM, NO_x and

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benzene, but not for VOCs like acetaldehyde and formaldehyde that are explicitly associated with indoor sources [44]. In this case, the levels of exposure are associated with the presence of building materials and furniture containing the respective compounds and the air exchange levels of the respective microenvironment. Air exchange rate is a key determinant of indoor air pollution, affecting both the processes of outdoor infiltration (e.g. outdoor PM infiltrate indoors) or accumulation of compounds emitted indoors (e.g. emission of formaldehyde from furniture). In practice, for compounds that significant sources occur both outdoors and indoors, indoor levels are defined by the overall interaction of indoor and outdoor air contamination [45]. Finally, regarding the mixture effects, people are always exposed to the mixture of PAHs and not to a single compound, although there are differences in the relative composition based on their origin, which is also reflected in their toxicity [46]. An efficient way to deal with the characterisation of these mixtures is the use of the Toxic Equivalent Quotient (TEQ), where for every component of the mixture, a Toxic Equivalent Factor (TEF) is given, based on its relative toxicity to benzo[a]pyrene; the overall toxicity of the mixture (TEQ) is the sum of the individual components concentration multiplied to the respective TEF. With regard to the quaternary mixture of benzene, toluene, ethylbenzene and xylene (BTEX), interactions have been identified at the level of metabolism (competitive inhibition acting upon the same CYP substrate). Thus, co-exposure to the BTEX mixture results in slower metabolism of benzene, compared to the metabolic rate of benzene when individual exposure to that substance occurs [47].

18.1.4 Policy relevance

PAHs are regulated on the basis of the National Emission Ceilings Directive 2001/81/EC. Moreover, Regulation (EU) 1272/2013 on PAHs in articles for supply to the general public, amended entry 50 of Annex XVII to REACH. According to this regulation, the use of PAHs has been restricted by a limit of 1 mg/kg (0,0001 % by weight) of BaP and 10 mg/kg (0,001 % by weight) for each of 8 PAHs for extender oils used for the production of tires or parts of tires. This regulation entered into force in January 2010. In addition, subject to the detailed scope of the restriction, a limit of 1 mg/kg is established for the rubber and plastic parts of many types of consumer articles. In the case of toys and childcare articles the limit is lowered to 0.5 mg/kg for each of 8 carcinogenic PAHs. The restriction entered into force in December 2015. Anthracene oil and coal tar pitch are included in the 6th recommendation of the European Chemicals Agency, of 1 July 2015 for the inclusion of substances in Annex XIV to REACH.

The main policy instrument regarding air pollutants within the EU is the Ambient Air Quality Directive [26, 27] and the National Emission Ceilings (NEC) Directive [48]. In 2011-2013 the Commission conducted a review of the EU air policies which resulted in the adoption of the Clean Air Policy Package in which the EU proposed a Clean Air Programme for Europe, updating the 2005 Thematic Strategy on Air Pollution in order to set new objectives for EU air policy for 2020 and 2030. The main legislative instrument towards 2030 objectives of the Clean Air Programme is Directive 2016/2284/EU on the reduction of national emissions of certain atmospheric pollutants which entered into force on 31 December 2016. This Directive sets national reduction commitments for the five pollutants (sulphur dioxide, nitrogen oxides, volatile organic compounds, ammonia and fine particulate matter) responsible for acidification, eutrophication and ground-level ozone pollution which leads to significant negative impacts on human health and the environment.

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Table 18-1: Legislative framework regarding PAHs and other air pollutants

Legislative reference	Matrix	ML30 (Y/N)	Compound
Commission Regulation (EC) No 1881/2006 Amended by Commission Regulation (EU) No 835/2011	Food	N31	16 EPA PAHs (mentioned as generic carcinogenic PAHs at point 58) Not included in other lists: Acenaphthene, Acenaphthylene, Anthracene, Fluoranthene, Fluorene, Naphthalene, Phenanthrene, Pyrene 15+1 EU PAHs: Benzo[a]anthracene, Benzo[b]fluoranthene, Benzo[j]fluoranthene, Benzo[k]fluoranthene, Benzo[c]fluorene, Benzo[ghi]perylene, Chrysene, Cyclopental[cd]pyrene, Dibenzo[a,h]anthracene, Dibenzo[a,e]pyrene, Dibenzo[a,h]pyrene, Dibenzo[a,i]pyrene, Dibenzo[a,l]pyrene, Indeno[1,2,3-cd]pyrene, 5-Methylchrysene
		Y	Benzo[a]pyrene plus the sum of the 4 marker PAHs (Benzo[a]pyrene, Benzo[a]anthracene, Benzo[b]fluoranthene and Chrysene)
Commission Regulation (EC) No 333/2007 Amended by Commission Regulation (EU) No 836/2011	Food	N	Benzo[a]pyrene Plus Benzo[a]anthracene, Benzo[b]fluoranthene and Chrysene
Commission Recommendation (2005/108/EC) of 4 February 2005 on the further investigation into the levels of polycyclic aromatic hydrocarbons in certain foods	Food	N	15 SCF PAHs: Benzo[a]pyrene, Benzo[a]anthracene, Benzo[b]fluoranthene, Benzo[j]fluoranthene, Benzo[k]fluoranthene, Benzo[c]fluorene, Benzo[ghi]perylene, Chrysene, Cyclopental[cd]pyrene, Dibenzo[a,h]anthracene, Dibenzo[a,e]pyrene, Dibenzo[a,h]pyrene, Dibenzo[a,i]pyrene, Dibenzo[a,l]pyrene, Indeno[1,2,3-cd]pyrene, 5-Methylchrysene
Commission Regulation (EC) No 672/2006	Primary Smoke products	N	15 SCF PAHs, Benzo[a]pyrene, Benzo[a]anthracene
Regulation (EC) No 2065/2003 of the European Parliament and of the Council	Primary Smoke products	Y	Benzo[a]pyrene, Benzo[a]anthracene
Directive 2000/76/EC of the European Parliament and the Council	Emissions from incineration plants	N	PAHs (Mentioned as carcinogenic compounds that might be subject to limitations in Member States' regulations)
Decision No 2455/2001/EC of the European Parliament and the Council	Water	N	PAHs (Annex: Identified as priority hazardous substance), Benzo[a]pyrene, Benzo[b]fluoranthene, Benzo[ghi]perylene, Benzo[k]fluoranthene, Indeno[1,2,3-cd]pyrene

³⁰ Maximum level (Y=yes; N=no)

³¹ Benzo[a]pyrene is considered a marker for PAHs

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Legislative reference	Matrix	ML30 (Y/N)	Compound
ANNEX XVII to REACH restriction Entry 50 Restrictions on the manufacture, placing on the market and use of certain dangerous substances, mixtures and articles	Oil, Toys, Articles, Tyres	Y	8 PAHs (mentioned as generic carcinogenic PAHs): Benzo[a]pyrene, Benzo[e]pyrene, Benzo[a]anthracene, Chrysen, Benzo[b]fluoranthene, Benzo[j]fluoranthene, Benzo[k]fluoranthene, Dibenzo[a,h]anthracene
Directives regulating ambient air quality (2008/50/EC, 2004/107/EC)	Air	Y	PM, O ₃ , NO ₂ , SO ₂ , CO, Bap
Directives regulating emissions of air pollutants (2001/81/EC)	Air	Y	NO ₂ , SO ₂ , NMVOC
Directive regulating ambient air benzene 2000/69/EC	Air	Y	Benzene

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Regulation on air pollutants is driven to a large extent by the data available with regard to their adverse health effects. Data come from the integration of epidemiological, controlled clinical studies, and animal toxicology. Epidemiological studies show statistical associations between health outcomes and exposure but they cannot establish a definite cause-effect relationship. However, the utility of toxicological studies lies in the possibility they provide to establish this relationship [49]. The European Union has developed an extensive body of legislation, based on the outcome of these studies, in order to establish health-based standards and objectives for a number of pollutants present in the air. Each air quality standard has two elements: the maximum acceptable concentration and the period of time period over which the concentration is averaged. During the 1990s, there was a great surge of reports on time-series-based studies of associations between daily ambient air pollutant concentrations and daily rates of mortality and hospital admissions for respiratory diseases [50]. In terms of morbidity, there has been a rapid growth of the literature showing associations between air pollutants concentrations and exacerbation of asthma, increased symptom rates and decreased respiratory function. The statistical techniques used by modern epidemiologists have seen considerable evolution. The result was that associations were found implying that far lower levels of pollutants were possibly adversely affecting human health on a wide scale [51, 52]. In summary, epidemiological studies so far have found associations between short-term changes in particulate air pollution and acute mortality (cardiovascular and respiratory related) and acute morbidity (hospital admissions, emergency room visits, exacerbation of asthma, respiratory symptoms, lung function measures, restricted activity days in workers, and school absences). These studies have associated ill health effects with increases in PM₁₀ by 10 µg/m³ and in PM_{2.5} by 1 µg/m³ over the previous day levels indicating thousands of annual deaths and other adverse effects [52]. Epidemiological evidence is overwhelming and strong enough on its own to support a particular standard (especially as results are replicated in several independent studies performed in different jurisdictions). Yet, toxicology offers the possibility to validate coherent mechanisms underlying the evidence upon which the standard is based. Toxicological studies also provide a basis for examining specific components within the complex ambient air mixture, such as PM; thereby they provide a basis for identifying the most toxic components of ambient air.

18.1.5 Technical aspects

Relevant individual PAHs to biomonitor, where feasible via their specific metabolites, include:

- ▶ 8 carcinogenic PAHs in entry 50 of Annex XVII to REACH: Benzo[a]pyrene, Benzo[e]pyrene, Benzo[a]anthracene, Chrysen, Benzo[b]fluoranthene, Benzo[j]fluoranthene, Benzo[k]fluoranthene and Dibenzo[a,h]anthracene
- ▶ 16 USEPA priority PAHs, included in numerous EN and national standards:
 - Naphthalene (CAS No. 91-20-3); Acenaphthene (CAS No.83-32-9); Acenaphthylene (CAS No.208-96-8); Fluorene (CAS No.86-73-7); Anthracene (CAS No.120-12-7); Phenanthrene (CAS No. 85-01-8); Fluoranthene (CAS No.206-44-0); Pyrene (CAS No.129-00-0); Benzo(a)anthracene (CAS No.56-55-3); Chrysene (CAS.No.218-01-9); Benzo(b)fluoranthene (CAS No. 205-99-2); Benzo(k)fluoranthene (CAS No.207-08-9); Benzo(a)pyrene (CAS No.50-32-8); Indeno(1,2,3-cd)pyrene (CAS No.193-39-5); Dibenzo(ah)anthracene (CAS No.53-70-3); Benzo(ghi)perylene (CAS No.191-24-2)
- ▶ Potentially also alkylated PAHs: 7,12-dimethylbenzo(a)anthracene; 1-methylphenanthrene; 2,3,5-trimethylnaphthalene; 1-methylnaphthalene; 2-methylnaphthalene and 2,6-dimethylnaphthalene.

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To study the exposure to PAHs, urinary mono-hydroxylated PAHs (OH-PAHs), a group of PAH metabolites, are commonly used as biomarkers (39). Among the OH-PAHs, 1-hydroxypyrene (1-PYR) is the most commonly used PAH biomarker in both occupational as well as in the general population from various countries (40).

From the technical point of view, methods already exist for the determination of some PAHs (such as BaP) in urine. Further methodological developments may be necessary however; solutions to this may be found by the European Human Biomonitoring Initiative cost-effectively. Considering that exposure to PAHs may occur from multiple sources and through multiple exposure routes, further understanding on the determination of the overall exposure levels is necessary. HBM information would be extremely useful in determining the overall exposure of the general population or of sensitive sub-populations, particularly children and specific target groups, to carcinogenic PAHs. HBM data would also help us determine whether the existing restrictions and limitations (in articles, in certain foods, in water, in ambient air) have a positive effect in reducing exposure to this ubiquitous family of chemicals or not. Finally, the HBM4EU work can also be very relevant in assessing worker exposure to these chemicals in certain activities (petrochemical plants, manufacture of anodes, etc.). With regard to the other air pollutants, actual biomarkers of exposure have been established only for benzene. These include either major benzene metabolites such as S-phenylmercapturic acid (S-PMA) and trans,trans-muconic acid (t,t-MA). However, due to their low sensitivity in common environmental settings, unmetabolised urinary benzene has also been suggested as a low exposure sensitive biomarker [53, 54]. With regard to other air pollutants, at the moment there are no well-established exposure biomarkers. Previous efforts have associated exposure to high levels of exposure to SO₂ with S-sulfonates in nasal lavage [55], and exhaled breath CO [56], while in the case of the main air pollutants exposure is usually associated with markers of inflammation [57].

18.1.6 Societal concern

PAHs are ubiquitous pollutants frequently found in a variety of environments such as oil, toys, food and atmosphere, increasing the exposure of humans to this chemical carcinogen even if in low concentrations. Due to their widespread distribution, the environmental pollution due to PAHs has aroused global concern. Many PAHs and their epoxides are highly toxic, mutagenic and/or carcinogenic to humans. Increased incidences of lung and skin cancers are associated with exposure to PAHs.

Benzene has been recognised as a carcinogen and the latest years focus is on the effects of prolonged exposure to low environmental levels. Although, the C₆H₆ emissions have declined sharply across the European Countries since the introduction of the Fuel Quality Directive [58], there is still concern raised from studies which show that the atmosphere around petrol stations contains significantly higher levels of benzene [59]. This has been strengthened by epidemiological studies, showing that children living close to a gasoline station, are subject to higher risk of leukemia [60].

PM is a widespread air pollutant, present wherever people live. The health effects of PM_{2.5} even at relatively low concentrations on health are significant. Effective management of air quality aiming to achieve low levels is necessary in order to minimise health risks. There is evidence that decreased levels of particulate air pollution following a sustained intervention result in health benefits for the population assessed.

These benefits come into place with almost any decrease in the PM levels. In many European Counties the PM_{2.5} concentrations, on average, tended to go down in the last decade [25]. However, in countries such as Croatia, Greece, Hungary and Spain a change in residential fuel consumption practices resulted in an increased use of biomass since 2005. This could be the

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combined result of the economic recession and/or the implementation of local policies such as incentivising the installation of biomass stoves in newly built or refurbished homes. As a result, biomass burning has turned into a major contributor to the atmospheric PM levels during wintertime [61] for a number of countries and major urban centers in the EU.

Overall, the importance of poor air quality has been recently highlighted by WHO, where it is mentioned that worldwide, ambient air pollution contributes to 5.4% of all-cause mortality [62].

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18.2 Categorisation of Substances

Table 18-2: Substances included in the substance group, listed according to availability of toxicology and human biomarker data, in category A, B, C, D, E substances (see general introduction)

Category	Abbrev./ Acronym	Systematic name	CAS No.	Regulation
A	NO2	Nitrogen dioxide	10102-44-0	Directive 2008/50/EC
A	SO2	Sulphur dioxide	7446-09-5	Directive 2008/50/EC
A	O3	Ozone	10028-15-6	Directive 2008/50/EC
A	CO	Carbon monoxide	630-08-0	Directive 2008/50/EC
B		Acenaphthene	83-32-9	According to the notifications provided by companies to ECHA in REACH registrations no hazards have been classified.
B		Acenaphthylene	208-96-8	
B		Antracene	120-12-7	Substance of very high concern (SVHC) and included in the candidate list for authorisation.
B	BaA	Benzo(a)anthracene	56-55-3	Entry 50 of Annex XVII to REACH
B	BaP	Benzo(a)pyrene	50-32-8	Entry 50 of Annex XVII to REACH
B	BbFA	Benzo(b)fluoranthene	205-99-2	Entry 50 of Annex XVII to REACH
B	BeP	Benzo(e)pyrene	192-97-2	Entry 50 of Annex XVII to REACH
B		Benzo(ghi)perylene	191-24-2	According to the classification provided by companies to ECHA in CLP notifications this substance is very toxic to aquatic life and is very toxic to aquatic life with long lasting effects.
B	BjFA	Benzo(j)fluoranthene	205-82-3	Entry 50 of Annex XVII to REACH
B	BkFA	Benzo(k)fluoranthene	207-08-9	Entry 50 of Annex XVII to REACH

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Category	Abbrev./ Acronym	Systematic name	CAS No.	Regulation
B		Dibenzo(ah)anthracene	53-70-3	Entry 50 of Annex XVII to REACH
B		Fluoranthene	206-44-0	According to the classification provided by companies to ECHA in CLP notifications this substance is very toxic to aquatic life, is very toxic to aquatic life with long lasting effects, is harmful if swallowed and causes serious eye irritation
B		Fluorene	86-73-7	According to the classification provided by companies to ECHA in REACH registrations this substance is very toxic to aquatic life and is very toxic to aquatic life with long lasting effects. ECHA has no data from registration dossiers on the precautionary measures for using this substance.
B		Chrysene/Benzo(a)phenanthrene	218-01-9	Entry 50 of Annex XVII to REACH
B		Indeno(123-cd)pyrene	193-39-5	According to the classification provided by companies to ECHA in CLP notifications this substance is suspected of causing cancer.
B		Naphthalene	91-20-3	According to the harmonised classification and labelling (CLP00) approved by the European Union, this substance is very toxic to aquatic life, is very toxic to aquatic life with long lasting effects, is harmful if swallowed and is suspected of causing cancer. Substance included in the Community Rolling Action Plan (CoRAP).
B		Phenanthrene	85-01-8	According to the classification provided by companies to ECHA in CLP notifications this substance is very toxic to aquatic life, is very toxic to aquatic life with long lasting effects and is harmful if swallowed.
B		Pyrene	129-00-0	According to the notifications provided by companies to ECHA in REACH registrations no hazards have been classified.
B		1-Methylnaphthalene	90-12-0	According to the classification provided by companies to ECHA in CLP notifications this substance may be fatal if swallowed and enters airways, is toxic to aquatic life with long lasting effects and is harmful if swallowed. ECHA has no data from registration dossiers on the precautionary measures for using this substance.
B		1-Methylphenanthrene	832-69-9	According to the classification provided by companies to ECHA in CLP notifications this substance is very toxic to aquatic life, is very toxic to aquatic

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Category	Abbrev./ Acronym	Systematic name	CAS No.	Regulation
				life with long lasting effects, is harmful if swallowed and is suspected of causing cancer.
B		2,6-Dimethylnaphthalene	581-42-0	According to the classification provided by companies to ECHA in CLP notifications this substance is very toxic to aquatic life and is very toxic to aquatic life with long lasting effects
B		2-Methylnaphthalene	91-57-6	According to the classification provided by companies to ECHA in CLP notifications this substance is toxic to aquatic life with long lasting effects and is harmful if swallowed. ECHA has no data from registration dossiers on the precautionary measures for using this substance.
B		7.12-Dimethylbenz(a)anthracene	57-97-6	According to the classification provided by companies to ECHA in CLP notifications this substance may cause cancer and is harmful if swallowed.
B	235TMNPT	2,3,5-trimethylnaphthalene	2245-38-7	According to the classification provided by companies to ECHA in CLP notifications this substance is very toxic to aquatic life, is very toxic to aquatic life with long lasting effects and is harmful if swallowed
B		Benzene	71-43-2	Entry 5 of Annex XVII to REACH
B		Toluene	108-88-3	Entry 48 of Annex XVII to REACH
B		Ethylbenzene	100-41-4	According to the harmonised classification and labelling (ATP06) approved by the European Union, this substance may be fatal if swallowed and enters airways, is a highly flammable liquid and vapour, is harmful if inhaled and may cause damage to organs through prolonged or repeated exposure.
B		Xylene	1330-20-7	According to the harmonised classification and labelling (CLP00) approved by the European Union, this substance is a flammable liquid and vapour, is harmful in contact with skin, is harmful if inhaled and causes skin irritation. Substance included in the Community Rolling Action Plan (CoRAP).
B		o-Xylene	95-47-6	According to the harmonised classification and labelling (CLP00) approved by the European Union, this substance is a flammable liquid and vapour, is harmful in contact with skin, is harmful if inhaled and causes skin irritation. Substance included in the Community Rolling Action Plan (CoRAP).

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Category	Abbrev./ Acronym	Systematic name	CAS No.	Regulation
B		m-Xylene	108-38-3	According to the harmonised classification and labelling (CLP00) approved by the European Union, this substance is a flammable liquid and vapour, is harmful in contact with skin, is harmful if inhaled and causes skin irritation. Substance included in the Community Rolling Action Plan (CoRAP).
B		p-Xylene	106-42-3	According to the harmonised classification and labelling (CLP00) approved by the European Union, this substance is a flammable liquid and vapour, is harmful in contact with skin, is harmful if inhaled and causes skin irritation. Substance included in the Community Rolling Action Plan (CoRAP).
B		Formaldehyde	50-00-0	According to the harmonised classification and labelling (ATP06) approved by the European Union, this substance is toxic if swallowed, is toxic in contact with skin, causes severe skin burns and eye damage, is toxic if inhaled, may cause cancer, is suspected of causing genetic defects and may cause an allergic skin reaction. Substance included in the Community Rolling Action Plan (CoRAP).
B		Acetaldehyde	75-07-0	According to the harmonised classification and labelling (CLP00) approved by the European Union, this substance is an extremely flammable liquid and vapour, causes serious eye irritation, is suspected of causing cancer and may cause respiratory irritation.
C		Biologicals (mould, pollen)		
C	PM	Particulate matter (PM1)		
C	UFP	Ultra-fine particles (UFP)		

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18.3 Policy-related questions

1. What is the current exposure of the EU population to PAHs?
2. What is the current exposure of different occupational groups?
3. Does exposure differ between countries? Why?
4. Is there an association between air quality and human exposure to PAHs??
5. Can we see a decline in exposure to the eight PAHs restricted under REACH?
6. Can HBM4EU data inform the development of legislation specifically targeting exposure to PAHs through ambient air?

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18.4 Research Activities to be undertaken

Table 18-3: Listing of research activities to be carried out to answer the policy questions summed up in 1.3

Policy question	Substance	Available knowledge	Knowledge gaps and activities needed
What is the current exposure of the EU population to PAHs?	PAHs	Data available for various European countries, but not for all of them – collected in different years	Collect, combine, harmonise and compare existing HBM and exposure data on PAHs relevant to the European population.
What is the current exposure of different occupational groups?	PAHs	Data available for various European countries, but not for various age groups	Collect, combine, harmonise and compare existing HBM and exposure data on PAHs and compare the data between different countries and population groups. Establish reference values for selected PAHs parent metabolites in urine for general population (adults/children, smokers/non-smokers) and for worker's populations (smokers/non-smokers).
Is there an association between air quality and human exposure to PAHs	PAHs	Limited data available for various European countries, lack of continuous monitoring	Collect, combine, harmonise and compare existing and new HBM and exposure data on PAHs and associate the data with air pollution levels.
Can we see a decline in exposure to the eight PAHs restricted under REACH?	PAHs	Limited data available for various European countries, lack of continuous monitoring	Collect, combine, harmonise and compare existing and new HBM and exposure data on PAHs and compare the data before and after the implementation of the REACH restriction.
Does exposure differ between countries? Why?	PAHs	Limited data available for various European countries	Collect, combine, harmonise and compare existing and new HBM and exposure data on PAHs and compare the data between different countries and associate these with exposure determinants.
Can HBM4EU data inform the development of legislation specifically targeting exposure to PAHs through ambient air?	PAHs		Collect new harmonised data that will fill the gaps related to various spatiotemporal scales and different population groups and associate these with exposure to PAHs through air

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18.5 Results Report

Table 3: Short overview of results of the activities carried out within HBM4EU to answer the policy questions with reference to corresponding deliverables

Policy Question		Short Summary of Results
No	Question	<i>Extract the main findings of the deliverable that answers (part) of the policy question, mention the deliverable</i>
1	What is the current exposure of the EU population to PAHs?	In WP12, exposure to PAHs was addressed using the data collected from available HBM data regarding 1-OH-pyrene. 1-OH-pyrene is a major metabolite of pyrene and is representative for the PAHs mixtures, while it is the metabolite that is commonly measured in the majority of PAH-related HBM studies. A PBTK model was parameterised and validated in Task 12.1 and it was coupled with the exposure reconstruction algorithms developed in Task 12.2. Based on the existing HBM data available at the moment, the median value of pyrene exposure ranges between 0.025 µg/kg_bw/d for non-smokers in Belgium to 0.240 µg/kg_bw/d for smokers in Netherlands. For most of the countries, median daily intake is around 0.050 µg/kg_bw/d, however, it has to be noted that, as described above, the bio samples had not been collected in the same year, while analyses were performed by different laboratories, thus, hampering the overall intercomparison; these estimates will be updated, upon the aligned study result will be available (AD12.5).
2	What is the current exposure of different occupational groups?	Exposure to the various occupational groups varies based on the specific activities of the related occupational sectors. The highest intake estimates were identified in soil remediation workers (in the range of 0.981 to 1.284 µg/kg_bw/d), followed by asphalt workers (0.093 to 0.325 µg/kg_bw/d) and workers in aluminum and rubber industry (0.035 to 0.100 µg/kg_bw/d). The lowest intake levels were identified to waste incinerator workers (0.004 to 0.104 µg/kg_bw/d), which is the only reported sector occupying both males and females. On the contrary, in all other sectors (soil remediation workers, asphalt workers, workers in aluminum and rubber industry) only males are being occupied and a differentiation on their intake results from their smoking habits, the time of their shift (pre shift, end of shift, post shift, next pre shift) and the age groups. The highest intake levels were related to soil remediation workers (1.284 µg/kg_bw/d) during the next pre shift, where pre shift and end of shift reported lower intakes (0.981 and 1.249 µg/kg_bw/d, respectively). For asphalt workers the highest intake was reported in the post shift and the specific age range of 35-52 (all workers were non-smokers). For workers in the aluminum and rubber industries, the lowest intake was reported for non-smokers (0.035 µg/kg_bw/d) comparing to smokers who exhibited a considerably higher intake (0.065 µg/kg_bw/d) (AD12.5).
3	Is there an association between air quality and human exposure to PAHs?	Dietary exposure dominates exposure to PAHs (contributing to almost 90 %) of daily intake, while the contribution of inhalation is lower (about 10%), except for the cases where significant sources of inhalation exposure such as the proximity to industrial hot spots, heavily trafficked roads, biomass emissions, as well as smoking; smokers have consistently higher exposure levels to pyrene, resulting to daily intake of between 0.015 to 0.150 µg/kg_bw/d. Regarding hot spots, it is expected that they result in higher pyrene concentrations in the range of 0.005 to 0.01 µg/kg_bw/d. (AD12.5)
4	Does exposure differ between countries? Why?	The difference in intake levels among the various countries are mostly explained by the differences in dietary intake, which is the result of increased soil contamination and dietary patterns (frequency of eating smoked food) and to a smaller extent to difference in air pollution levels. More in detail, based on the available HBM data available so far, the highest intake levels were calculated in Netherlands (0.073 to 0.245 µg/kg_bw/d) followed by Germany (0.019 to 0.125 µg/kg_bw/d) and Greece (0.060 to 0.065 µg/kg_bw/d), Denmark (0.041 to 0.095 µg/kg_bw/d), Czech (0.053 µg/kg_bw/d), France (0.022 to 0.078 µg/kg_bw/d) and Italy (0.041 to 0.059 µg/kg_bw/d), Spain (0.035 µg/kg_bw/d) and Belgium (0.029 µg/kg_bw/d). The lowest intake levels were reported in Sweden (0.013 to 0.036 µg/kg_bw/d). It has to be noted that several exposure modifiers such as age, smoking status and exposure to secondhand smoke, as well as residential location have been identified as key factors affecting the overall intake levels. In

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Policy Question		Short Summary of Results
		Netherlands, Italy, France and Sweden the intake levels of smokers have been identified much higher compared to the ones of non-smokers (0.245 and 0.073 µg/kg_bw/d, 0.059 and 0.041 µg/kg_bw/d, 0.078 and 0.022 µg/kg_bw/d and 0.036 and 0.013 µg/kg_bw/d, respectively). In Germany the highest intake levels were reported for children of 5-8 years old, living near industrial hot spots (0.125 µg/kg_bw/d) while for children of the same ages living away from industrial hot spots the intake levels were much lower (0.064 µg/kg_bw/d). This is explained by the higher multimedia contamination in the area and the higher contribution to intake of both soil ingestion and ambient air inhalation. In Greece, living nearby areas with traffic congestion, the intake levels were higher than in urban areas free of traffic (0.065 and 0.060 µg/kg_bw/d, respectively). In Denmark the highest intake levels were reported for bus drivers of 27-60 years of age (0.095 µg/kg_bw/d) while the lowest ones were reported for people working in rural areas (0.041 µg/kg_bw/d) (AD12.5). However, the reason why differences are reported among the various countries will be further explored when the latest HBM data will be available and the statistical analysis in WP10 will have been completed.
5	Can we see a decline in exposure to the eight PAHs restricted under REACH?	Exposure to PAHs occurs through multiple pathways and routes. This also pertains for the 8 PAHs (benzo[a]pyrene, benzo[e]pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[j]fluoranthene, benzo[k]fluoranthene and dibenzo[a,h]anthracene) restricted under REACH. Restrictions from REACH are expected to affect the contribution of exposure related mainly to consumer products. It is also likely that the restriction of use will result in a reduction in the overall tonnage that will be reflected in the soil levels, which in turn will be reflected in the food chain and the dietary intake. However, to identify a potential decline, a trend analysis is required, which in turn requires the acquisition of the completion of the statistical analysis of existing data (from Tasks 10.3 and 10.4) and the collection of new data (Task 8.3: Targeted new field work with EU added value). At the moment there are not enough data to support this hypothesis.
6	Can HBM4EU data inform the development of legislation specifically targeting exposure to PAHs through ambient air?	At the moment the EU Scientific Committee on Occupational Exposure Limits (SCOEL) has provided a biological guidance value (BGV) for PAH mixtures containing benzo[a]pyrene equal to 0.5 µg/L hydroxypyrene in urine. It has to be noted that the limit values recommended by SCOEL have not been implemented into legislation by the Member States. Based on the work that will be carried out in WP5, EU HBM-HBGV will be derived on the basis of toxicological studies. The values represent the concentration of a substance in human biological material below which there is no risk for adverse health effects and, consequently, no need for action. Hence, they are an important tool to easily assess whether the exposure of a population/subpopulation (e.g. reference values) is of health-relevance and whether policy actions are needed. These values will together with the result of WP10 be used also to address this research question. In addition, input will be provided from the work done in WP12, towards the association of the dose of toxic metabolites in the target tissue, with the observed HBM levels. In addition, work on exposure reconstruction of PAHs has indicated that most of exposure to PAHs comes from dietary sources rather than ambient air pollution, which is contributing for almost 10% of the overall exposure to diet (AD12.5).

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19 Prioritised substance group: Perfluorinated substances (PFAS)

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19.1 Background information

19.1.1 Introduction

Per- and polyfluoroalkyl substances (PFASs) have been in use since the 1950ies as ingredients of intermediates of surfactants and surface protectors for assorted industrial and consumer applications. Within the past decade, several long-chain perfluoroalkyl acids have been recognised as extremely persistent, bioaccumulative and toxic. Many have been detected globally in the environment, biota, food items, and in humans (OECD, 2015, 2018). It has been recognised more recently that shorter chain PFASs increasingly used as alternatives are also very persistent and thus very mobile in the environment, leading to ground water contamination now and presumably in future. To date many known and unknown alternatives of the so far regulated PFASs are used worldwide leading to environmental contamination und increasing human body burdens.

19.1.2 Hazardous properties

PFASs bind to proteins and partition to phospholipids. The elimination kinetics are highly species dependent, with humans showing the longest half-lives of up to e.g. 8.5 years for perfluorohexane sulfonic acid (PFHxS). An estimated elimination range of 10.1 to 56.4 years – median 15.3 years for chlorinated polyfluoroalkyl ether sulfonic acids [Cl-PFESAs] has been reported in Shi et al., 2016. The CLP human health hazard classifications of the different substances are depicted in table 1. Substances which are best-known – perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) – are classified as carcinogenic (Carc. 2, suspected human carcinogens, such as kidney and testicular), toxic for reproduction (Repr. 1B, presumed human reproductive toxicants; Lact., may cause harm to breast-fed children), toxic to specific target organs after repeated exposure (STOT RE 1) and acute toxic (Acute Tox. 3-4) for different exposure routes. PFOS and PFOA belong to the so called long-chain perfluorinated compounds, which refers to perfluorocarboxylic acids with carbon chain lengths of 8 and higher, including PFOA; perfluoroalkyl sulfonates with carbon chain lengths of 6 and higher, including PFHxS and PFOS; and precursors of these substances that may be produced or may be present in products. It could be shown that in product samples the detected individual PFAS constituted only a very minor part of the total organic fluorine (TOF), illustrating large data gaps in the current knowledge which PFASs that are being used in these products (Borg, 2017).

Several long-chain compounds beside PFOS and PFOA have also been identified as toxic to reproduction; further endpoints concern carcinogenicity, liver toxicity, neurotoxicity and

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immunotoxicity. Whether numerous other non-regulated PFASs show similar toxicity is currently less well established. In many cases data availability is poor and therefore no classification is possible. However, persistence is assumed to concerns largely all PFAS by reason of the extreme strength and stability of the carbon-fluorine bonds.

For PFOS and PFOA adverse effects on thyroid metabolism and lipid metabolism have been reported in a multitude of epidemiological studies suggesting endocrine disrupting potential (Barry et al., 2013, HBM4EU, Deliverable 14.2, 2018).

Additional concerns include increased risk of miscarriage, reduced birth weight, increased weight in adult life, and reduced fertility among offspring as a result of early life exposures (Halldorsson et al., 2012; Jensen et al., 2015; Joensen et al., 2013; Timmermann et al., 2014). Postnatal exposures have also been associated with thyroid hormone imbalances and reduced immune response to vaccination (Grandjean and Budtz-Jørgensen, 2013). The US National Toxicology programme has listed both, PFOA and PFOS, as presumed to be an immune hazard to humans (NTP, 2016). Immunotoxicity has been also identified as most sensitive endpoint in humans (EFSA, 2020).

Grandjean and Clapp (2015) documented carcinogenicity, immunotoxicity and developmental toxicity of PFOA and highlighted the endocrine disrupting effects. In a Danish mother- child cohort prenatal exposure to perfluoroalkyl substances lead to reduction in anogenital distance in girls at 3 months of age (Lind et al., 2017).

A systematic review on health effects of PFAS exposure and childhood health outcome observed generally consistent evidence for PFAS' association with dyslipidemia, immunity including vaccine response and asthma, renal function, and age at menarche (Rapazzo et al., 2017).

A comparison of birth outcomes in a PFAS contaminated region in Italy with a less exposed population group showed a significantly increased risk for gestational diabetes, preeclampsia and small size for gestational age. Further biomonitoring data would be needed to confirm direct cause and effect (WHO, 2015).

Long chain PFASs (certainly PFOA, PFOS & PFHxS, and possibly others) are actively reabsorbed in the kidney and intestine. This active reabsorption varies and the determinants of the variation (e.g. renal function) may a) be a confounder in using serum levels as the exposure marker in analysing health effects, b) may make individuals more or less vulnerable to the adverse health effects and thus affect how health based limits are set, or c) may be a basis for identifying subgroups at extra risk.

Since PFOS and PFOA can still be measured in highest concentrations in biota and in humans, exerting similar toxic effects along with and similar to a range of long-chain PFASs measured in blood, together with a range of unidentified PFASs the possibility of mixture effects is very high.

19.1.3 Policy relevance

In June 2019, the European Council of Ministers has highlighted the widespread occurrence of PFAS in environment, products and humans and has called for an action plan for the elimination of all non-essential PFAS uses³². The European commission agreed on the need of regulatory and non regulatory actions, which is also in line with the chemicals strategy for sustainability (toxic-free EU environment) and the Green Deal. European member states have provided elements for an

³² <https://www.consilium.europa.eu/media/40042/st10713-en19.pdf>

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EU-strategy for PFASs, including various regulatory and non regulatory actions to the Commission³³.

Five European states including Germany, the Netherlands, Norway, Sweden and Denmark have agreed to prepare a joint REACH restriction proposal with the aim to limit the risks to human health and environment from the production and use of a wide range of PFAS for non essential uses. Therefore, a call of evidence is open until end of July 2020; information received will be used to refine the scope of the proposal and to analyse effectiveness and socio-economic impact of restriction options³⁴. Further, a restriction proposal (Annex XV dossier) will be prepared. The possible date of entry into force of the restriction is in 2025 expected (ECHA, 2020a).

The EEA has published a policy briefing on PFAS in 2019 (EEA, 2019).

REACH and Stockholm Convention

Previous regulatory actions within the European Union and elsewhere concern PFOS and its derivatives (POP regulation, Commission Regulation (EU) No 757/2010) PFOA and related substances are subject of a restriction of the manufacturing, marketing and use (EU 2017/1000). This will be replaced by a new restriction under the EU POPs regulation which will include more limited derogations following a decision of the Stockholm Convention (EEA, 2019). Certain per- and polyfluorinated substances can be degraded to persistent perfluorinated substances like PFOS or PFOA under environmental conditions or in humans and are therefore precursors. With the current regulations on PFOS and PFOA also these precursor substances are subject to the EU restrictions. Additional identities of PFOS- and PFOA-related substances can be found in ECHA (2014), Buck et al. (2011), Environment & Health Canada (2012), OECD (2011) or U.S. EPA (2006). OECD, 2018 refers to more than 4700 substances related to PFAS (OECD, 2018).

Several regulatory actions have been taken since 2012 by ECHA: A restriction proposal for long-chain PFCAs covering perfluorononan-1-oic acid (PFNA), nonadecafluorodecanoic acid (PFDA), hencosafluoroundecanoic acid (PFUnDA), tricosfluorododecanoic acid (PFDoDA), pentacosfluorotridecanoic acid (PFTrDA), heptacosfluorotetradecanoic acid (PFTDA), including their salts and precursors was submitted to ECHA mid 2017 (Germany, 2017).

For PFHxS, its salts and related substances Norway prepared a restriction proposal. The public consultation ended at 25 May 2020 (ECHA, 2020c).

At the 15th meeting of the Persistent Organic Pollutants Review Committee in October 2019, the risk management evaluation of PFHxS, its salts and related substances was adopted. It is recommended to consider these substances to be listed in Annex A of the Stockholm convention without specific exemptions (Stockholm Convention, 2019).

For PFHxA, its salts and related substances a restriction proposal was prepared by Germany. A 6 month consultation started end of March 2020 (ECHA, 2020b).

Several long-chain PFASs are also on the Candidate List of substances of very high concern (SVHC) under REACH: e.g.: PFDA and its sodium and ammonium salts (Reprotox. (57c) and PBT (57d)), nonadecafluorodecanoic acid, decanoic acid, nonadecafluoro-, sodium salt, ammonium nonadecafluorodecanoate, perfluorononan-1-oic-acid and its sodium and ammonium salts (Reprotox (57c)), perfluorononan-1-oic-acid, sodium salts of perfluorononan-1-oic-acid, ammonium salts of perfluorononan-1-oic-acid, ammonium pentadecafluorooctanoate (APFO) (Reprotox. (57c) and PBT (57d)), hencosafluoroundecanoic acid (C11-PFCA) (vPvB (57e)), and

³³ <https://www.regjeringen.no/contentassets/1439a5cc9e82467385ea9f090f3c7bd7/fluor---eu-strategy-for-pfass---december-19.pdf>

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heptacosafuorotetradecanoic acid (C14-PFCA) (vPvB (57e)). Other widely used substances are still under substance evaluation or are foreseen to be regulated under REACH, such as PFASs (PFHxS, PFBS), ADONA, 6:2 FTMA and several short-chain PFCAs (C₄-C₇). In 2019, GenX was added to the SVHC list based on its PMT properties posing threats to drinking water and environment. Additionally, several PFAS, including GenX are on the CoRAP for evaluation over the next years (EEA, 2019).

There is a lack of information on PFASs from imported articles as well as work on fluoropolymers and fluoroethers to clarify if those can be perceived as PFASs precursors (Pelthola-Thies, 2017).

To summarise: ECHA has worked on PFAS since 2012 with a substance-by-substance risk management approach. In 2014, ECHA moved to an approach that looks at PFAS subgroup-by-subgroup. This has also proven to be slow. So, there needs to be a more ambitious way of looking at a whole group of substances (Peltola-Thies, 2020). There are also efforts to restrict PFAS in firefighting foams and in textiles, upholstery, leather, apparel and carpets (TULAC) under REACH.

Other legislative measures

Revision of the drinking water directive

The EU drinking water directive (Council Directive 98/83/EC on the quality of water intended for human consumption) in force not includes limits for PFAS. However, during the ongoing revision of the directive, limit values for PFAS are suggested comprising 0.1 µg/L for the sum of specific PFAS considered to be an concern for drinking water (including 20 substances³⁵) as well as 0.5 µg/L for "PFAS total"³⁶. The member states may be then decide to use either one or both of the proposed limit values. It addition, technical guidelines regarding the analytical methods including limits of detection, parameter values and sampling frequency for monitoring of PFAS total and the sum of PFAS should be established (Council of the European Union, 2020).

Various PFASs are used as food contact materials (FCM) and also as flavouring in food, e.g. one of the flavourings currently approved under Regulation No 1334/2008 is a polyfluorinated organic chemical (FL16.119, N-(2-methylcyclohexyl)-2,3,4,5,6-pentafluorobenzamide).

There are voluntary agreements with industry in Canada or the USA to phase out PFASs C8 chemistry like the U.S. EPA Stewardship Programme³⁷.

PFASs have been recognised as an issue for concern under SAICM (Strategic International Approach to International Chemicals Management)³⁸. The OECD has established a web portal in order to facilitate information exchange among stakeholders³⁹.

On national level in Europe several states have set national limits for PFOS in e.g. water, soil (DK, DE, NL, SE), textiles (NO), and food contact materials (DK). In addition, several member states of the EU set limits in drinking water for specific PFAS and for specific groups of PFAS (EEA, 2019). In 2019 Denmark has announced a ban on paper and cardboard used in food contact materials by July 2020 (FCM, 2019).

³⁵ Perfluorobutanoic acid (PFBA), Perfluoropentanoic acid (PFPA), Perfluorohexanoic acid (PFHxA), Perfluoroheptanoic acid (PFHpA), Perfluorooctanoic acid (PFOA), Perfluorononanoic acid (PFNA), Perfluorodecanoic acid (PFDA), Perfluoroundecanoic acid (PFUnDA), Perfluorododecanoic acid (PFDoDA), Perfluorotridecanoic acid (PFTrDA), Perfluorobutanesulfonic acid (PFBS), Perfluoropentanesulfonic acid (PFPS), Perfluorohexanesulfonic acid (PFHxS), Perfluoroheptane sulfonic acid (PFHpS), Perfluorooctanesulfonic acid (PFOS), Perfluorononane sulfonic acid (PFNS), Perfluorodecane sulfonic acid (PFDS), Perfluoroundecane sulfonic acid, Perfluorododecane sulfonic acid, Perfluorotridecane sulfonic acid.

³⁶ "PFAS total" means the totality of per- and polyfluoroalkyl substances.

³⁷ <http://epa.gov/oppt/pfoa/pubs/stewardship/index.html>

³⁸ <http://www.saicm.org/EmergingPolicyIssues/Perfluorinatednbspc;Chemicals/tabid/5478/language/en-US/Default.aspx>

³⁹ http://www.oecd.org/ehs/pfc/#Purpose_of_Web_Portal

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19.1.4 Exposure characteristics

Trends in production volume/environmental concentrations

A minor part of the family of PFASs are perfluoroalkyl acids (PFAA), perfluoroalkylcarboxylic acids (PFCA), perfluoroalkane sulfonic acids (PFSA), compounds derived from perfluoroalkane sulfonyl fluoride (PASF), fluorotelomer (FT)-based compounds and per- and polyfluoroalkylether (PFPE)-based compounds. Another presumably major part are polymers (fluoropolymers (FPs), side-chain fluorinated polymers and perfluoropolyethers (PFPEs) (OECD, 2013). According to KEMI (2017) there are 2,817 PFASs on the market. For only 15 % of them adequate data are available; whereas for 40% data are missing (KEMI, 2017). Many fluorinated substances enter the EU through the import of articles (e.g. textiles) and for the most part these are not monitored (KEMI, 2015) providing an indirect exposure source. The lack of data concerns identification, use and exposure beside from toxicity and ecotoxicity. Among the new chemical groups, fluoro silicones, perfluoro polyethers and perfluoro alkanes are under discussion. Recent uses comprise surfactants, repellents, uses in textiles and in leather, paper and electronic industry, cosmetics, pesticides, lubricants, pharmaceuticals and printing (Fischer, 2017). For the large group of polymers no data are available at all, as polymers are not covered within REACH. However, there are concerns from the scientific point of view that at least some groups of polymers may also be degraded into persistent PFASs. For example fluorinated side-chains can be lost through ageing and environmental conditions.

Environmental behaviour: half-lives in environment/ transport

Perfluoroalkyl and perfluoroether moieties of PFASs are highly persistent under environmental conditions. All PFASs ultimately degrade into highly persistent end products. PFASs are ubiquitously detected in the environment. Contamination of the drinking water resources as environmental health thread has been reported for PFASs e.g from the Veneto Region in Italy (WHO, 2017) but also from Sweden (Banzhaf et al, 2017) and other European countries. Whereas most data are available for the small group of long-chain PFASs, non-reversible environmental exposure has to be considered for a by far larger group. Recent data demonstrate considerable exposure of alternatives such as GenX in the drinking water (e.g. Gebbink et al., 2017).

There are also concerns about short-chain PFASs, which are assumed to be less bioaccumulative but very persistent and mobile contaminants found in drinking water and food, including vegetables (Hedlund, 2016, Danish EPA, 2015).

Human-related exposure sources and uses, human exposure routes

Humans can be exposed directly (via diet, drinking water, consumer products, etc.) and indirectly through transformation of «precursor substances» such as polyfluoroalkyl phosphate esters (PAPs), fluorotelomer alcohols (FTOHs), fluorotelomer iodides (FTIs) and fluorotelomer acrylate monomers (FTAcS). These fluorotelomer-based substances biotransform to yield PFCAs, yet also form bioactive intermediate metabolites, which have been observed to be more toxic than their corresponding PFCAs (e.g. Rand et al., 2017). The precursor contribution to PFASs daily exposures was recently estimated for a high exposure scenario to contribute up to >50% to individual PFCAs like PFOA or PFDA, whereas it is considerable lower up to 10% for e.g. PFOS for a low exposure scenario (Gebbink et al., 2015).

Human Biomonitoring (HBM) data availability

Human exposures to PFASs have been reported in numerous studies in Europe and worldwide. Most of these studies were focused on blood or breast milk concentrations of PFOS and PFOA, while others also included PFBS, PFHxS, PFDS, PFBA, PFPeA, PFHxA, PFHpA, PFNA, PFDA, PFUdA, PFDoA, PFTTrDA, PFTeDA, FOSA, MeFOSA, N-EtFOSA, N-EtFOSAA and diPAP.

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Contrary, human exposure to e.g. 8:2 diPAP, 6:2 diPAP, 8:2 PAP, 6:2 PAP, PFDPA, PFOPA, PFHxPA or ADONA has been addressed to a small extent only; the majority of new fluorinated compounds that enter the market as replacements has not been measured in human matrices yet. Concerning PFOS the effectiveness evaluation under the UNEP Stockholm Convention concluded that for human matrices from Western Europe, Canada, Australia and Asia-Pacific countries levels seem gradually declining. Although PFOS is measured at low concentrations in human breast milk and is detected in higher concentrations in human blood, there are good correlations between the measurement results in these two matrices (UNEP, 2016).

There are major knowledge gaps on fluorinated alternatives currently used by industry; these knowledge gaps concern production volumes, use, fate and behaviour, and toxicity (Danish EPA, 2013; Wang et al., 2013, 2016, 2017). Known fluorinated alternatives can be categorised into two groups, namely [i] shorter-chain homologues of long-chain PFAAs and their precursors, and [ii] functionalised perfluoropolyethers (PFPEs), in particular perfluoroether carboxylic and sulfonic acids (PFECAs such as ADONA and GenX and PFESAs such as F-53 and F-53B) (Wang et al., 2015). Perfluoroalkyl phosphonic and phosphinic acids are also used as alternatives in certain applications. PFPAs are likely to be persistent and long-range transportable, whereas PFPiAs may be transformed to PFPAs and possibly PFCAs in the environment and in biota (Wang et al., 2016).

In environmental samples fluorotelomer-based substances were identified as the most relevant precursors of PFCAs based on the frequency of detection and the concentration of FTOHs, biotransformation intermediates (e.g. FTUCAs and FTCAs) and persistent biotransformation products (e.g. x:3 acids and PFCAs) (UBA, 2016).

EFSA has published two scientific opinions on PFAS, the first opinion concerns PFOS and PFOA, the second opinion focused on possible risks to human health from multiple PFASs, as they are often present in mixtures in the food chain (EFSA, 2018, 2020). The CONTAM panel has assessed the exposure of the European population and also of the available Human Biomonitoring data.

EFSA concluded on timetrends: *The general trends observed in the time-trend studies can be summarised as follows: (i) the concentrations of most PFASs and PFCAs increased from the early 1970s up to around the year 2000, (ii) the concentrations of PFOS and PFOA have in most studies been observed to decrease from approximately the year 2000, while in many studies PFNA, PFDA and PFUnDA have increased or at least remained stable during the same time period, (iii) variable trends have been reported for PFHxS, while no particular change has been reported for some of the PFASs that are present in low concentrations in humans. This is in line with the conclusions in a recent review on time trends of PFASs Land et al., 2018). Median values for the study medians were determined, referred to as median concentrations.*

And on exposure: *The most prominent PFAS in serum of adults was PFOS (64%), followed by PFOA (16%), PFHxS (5.6%) and PFNA (5.1%). For children, PFOS and PFOA contributed almost the same with 35.0% and 36.6% of the total, followed by PFNA (8.8%) and PFHxS (6.7%). For adults the median concentrations in serum or plasma were 7.7, 1.9, 0.67, 0.61, 0.30 and 0.28 ng/mL for PFOS, PFOA, PFHxS, PFNA, PFDA and PFUnDA, respectively, while the concentrations of the remaining PFASs were below 0.25 ng/mL. For children the median concentrations in plasma were 3.2, 3.3, 0.79, 0.60 and 0.30 ng/mL for PFOS, PFOA, PFNA, PFHxS and PFDA, respectively, while the concentrations of the remaining PFASs were below 0.25 ng/mL. Considerably higher concentrations have been observed for some individuals, including both occupationally exposed adults, and children and adults, which have experienced elevated exposure from e.g. contaminated drinking water.*

A recent publication addresses the occurrence of TFA trifluoroacetic acid (TFA) levels in the blood of Chinese people in concentrations almost as high as PFOA. 6:2 Cl-PFAES and TFA are detected

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with high levels in serum, second to PFOA and PFOS. A significant increase in 6:2 Cl-PFAES with age and BMI, and TFA with age are found. Positive associations of several PFASs with fasting glucose and HbA1c are observed (Duan et al., 2020).

An detailed investigation on existing European HBM studies were made within in the HBM4EU project: Deliverable D10.6 includes aggregated data of existing HBM data collections which were obtained through HBM4EU for different PFAS in toddlers aged 1-2 years and adults aged > 20 years in blood and breast milk, including the substances FOSA, N-EtFOSA, N-MeFOSA, PFBA, PFBS, PFDA, PFDoDA, PFDS, PFHpA, PFHpS, PFHxA, PFHxS, PFNA, PFOA, PFOS, PFPeA, PFTeDA, PFTrDA and PFUnDA (HBM4EU, 2020).

Further data from the aligned studies in European teenagers will become available as well as investigations from cohort studies.

Health based guidance values available for HBM data

Recently, the EFSA has reassessed the risk of PFAS: In 2018, EFSA concluded that a notable part of the European population is exposed to PFOS and PFOA via food leading to exceedances of the tolerable daily intake of up to 25 times. For the deviation of health-based guidance values, human epidemiological studies were used as basis. The identified critical effects include for PFOS the increase of total cholesterol levels in serum of adults, and the decrease in the antibody response at vaccination in children, and for PFOA also the increase in serum cholesterol in adults. In addition, reduced birth weight for PFOS and PFOA and increased prevalence of high levels of alanine aminotransferase (ALT) in serum for PFOA were considered.

Based on the available data EFSA proposed tolerable weekly intakes (TWI) of 13 ng/kg bw/week for PFOS and 6 ng/kg bw/week for PFOA in 2018. For both, the exposure of a considerable part of the European population exceeds those TWIs (EFSA, 2018). In 2020, EFSA published its draft scientific evaluation of the sum of four PFAS including PFOS, PFOA, PFNA and PFHxS. Based on human epidemiological data and data from animal studies, effects on the immune system was considered as the most critical for the assessment. EFSA set out a updated TWI for the sum of the four named PFAS of 8 ng/kg bw/week, which should protect also against other adverse effects observed in humans (EFSA, 2020).

Revision of the drinking water directive

As described above proposed limit values for PFAS are 0.1 µg/L for the sum of specific PFAS considered to be an concern for drinking water (including 20 substances⁴⁰) as well as 0.5 µg/L for "PFAS total"⁴¹.

Nevertheless, it has to be noted that when applying the proposed limit values the respective PFAS concentrations which could be uptaken via a drinking water consumption of 1-2 litre per day would exceed the risk-based tolerable weekly intake (of the sum of PFOS, PFOA, PFHxS and PFNA of 8 ng/kg bodyweight/day) derived by EFSA (EFSA, 2020) especially in children dramatically. For example: considering a PFAS concentration of 0,1 µg/L in drinking water, a child with a bodyweight of 10 kg and a daily drinking water consumption of 1 litre has a PFAS-intake of 700 ng/week. When

⁴⁰ Perfluorobutanoic acid (PFBA), Perfluoropentanoic acid (PFPA), Perfluorohexanoic acid (PFHxA), Perfluoroheptanoic acid (PFHpA), Perfluorooctanoic acid (PFOA), Perfluorononanoic acid (PFNA), Perfluorodecanoic acid (PFDA), Perfluoroundecanoic acid (PFUnDA), Perfluorododecanoic acid (PFDoDA), Perfluorotridecanoic acid (PFTrDA), Perfluorobutanesulfonic acid (PFBS), Perfluoropentanesulfonic acid (PFPS), Perfluorohexanesulfonic acid (PFHxS), Perfluoroheptane sulfonic acid (PFHpS), Perfluorooctanesulfonic acid (PFOS), Perfluorononane sulfonic acid (PFNS), Perfluorodecane sulfonic acid (PFDS), Perfluoroundecane sulfonic acid, Perfluorododecane sulfonic acid, Perfluorotridecane sulfonic acid.

⁴¹ "PFAS total" means the totality of per- and polyfluoroalkyl substances.

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applying the TWI of 8ng/kg bodyweight/week leading to a exposure of 80 ng/week, the uptake would be 8,75-times higher.

19.1.5 Technical aspects

Biomarkers available for parent compounds or metabolites in human matrices, and main characteristics of analytical methods (quantitative, semi-quantitative...)

Analytical targets for the analysis in biomonitoring studies can include the parent compound, its metabolite(s) and transformation product(s) or other chemical products formed in the body or the environment. Known PFASs were mostly analysed by high performance liquid chromatography coupled with tandem mass-spectrometry (HPLC-MS/MS). FTOH and FTOH precursors (FTMAC and PAPs) and their metabolites can be measured by targeted methods, by low or high resolution mass spectrometry. Methods for possibly cationic PFASs (such as betaines used e.g. in firefighting foams) can be analysed using specific methods used for environmental matrices. Analyses of FTMAC require derivatisation, followed by gas chromatography coupled with mass-spectrometry (GC-MS) analysis (Place and Field, 2012; Trier, pers. comm., 2017). In recent years, several studies on total fluorine (TF), inorganic fluorine (IF), extractable organic fluorine (EOF) and specific known PFASs in environmental and blood samples were conducted. Usually, TF, IF and EOF were fractionated and measured by combustion ion chromatography (CIC). It has been shown that PFOS was still the dominant PFAS contributing up to 90% to known PFASs in 30 blood samples sampled in three Chinese cities in 2004. PFOS, PFHxS, PFOSA, PFDODA, PFUnDA, PFDA, PFNA, PFOA, PFHpA, PFHxA contributed 33 to 85% to total EOF (Yeung et al., 2008).

In 2016, Yeung and Mabury (2016) investigated blood samples from China and Germany to identify concentrations of EOF and 52 specific PFASs including including PFASs, PFCAs, PFPAs, PFPiAs, FTSAa, PAPs, FTCAs/FTUCAs, di-SAmPAPs, FASAs, FOSAA and N-alkyl-FOSAA. PFASs represented the majority of EOF with decreasing contribution: 70% in 1982, 60% in 2003, 25% in 2009. Mass balance analysis between EOF, which provides an estimate of all fluorinated substances, and known quantifiable PFASs in human blood samples have shown the presence of unidentified organofluorides up to 80%. These findings suggest that other PFASs (e.g. precursor or intermediate compounds) might be significantly important (Yeung and Mabury, 2016). A detailed description of the study results can be found elsewhere (Miyake et al., 2007; Yeung et al., 2008; 2009, Yeung and Mabury 2016)

However, these methods may not allow distinguishing between PFASs exposure and fluorine based medication. This concern is particularly related to the fact that many pharmaceuticals may contain fluorinated moieties to make them more persistent in human bodies (Wang, pers. Comm., 2017).

In best of our knowledge, it is not feasible and reasonable to measure all relevant PFAA precursors due to a lack of an overview on which precursors are being produced and used and to which ones humans are exposed to at the moment. Considering that most precursors would be transformed into acids in human body, it would be an interesting approach to measure the “total oxidisable precursors” in human matrices. The “total oxidisable precursors” methods have been used to reflect the total exposure to PFAAs and PFAA precursors in a number of environmental samples. Due to its nature of radical reactions with a large, complex mixture, the methods may not easily or never be standardised and the results may not be reproducible. However, it might be a semi-quantitative indicator to demonstrate PFAAs exposure stemming from the variety of precursors (Wang, pers. Comm., 2017).

Further analytical methods to simultaneously analyse as many PFASs as possible should be developed (Wang et al., 2016).

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19.1.6 Societal concern

A recent study estimated the costs to society arising from PFAS exposure are high, with the annual health-related costs estimated to be EUR 52-84 billion across Europe in a recent study (Nordic Council of Ministers, 2019). The study notes that these costs are likely underestimated, as only a limited range of health effects (high cholesterol, decreased immune system and cancer) linked to exposure to a few specific PFAS were included in the estimates. In addition, PFAS pollution also affects ecosystems and generates costs through the need for remediation of polluted soil and water. Such costs are currently difficult to assess since information on the number and scale of sites contaminated with PFAS in Europe and on how PFAS impact ecosystems is lacking.

PFASs are widely used in society and be as a whole group a cause for concern. Individual PFASs or their degradation products are extremely persistent in the environment and it has been shown that several of them are very mobile, bioaccumulative and toxic, whereas for several others there is only some indication as scientific proof is lacking at present. Nevertheless, many PFASs, including fluorinated alternatives to long-chain PFASs, can be ubiquitously detected in the biotic and abiotic environment, in wildlife and in humans, even in remote regions such as the Arctic since several years. In several countries PFASs have been found in ground and drinking water (Domingo et al., 2012; KEMI, 2017).

Currently, there are several contamination cases known in different countries (e.g. in Germany, Sweden, Italy, Spain and The Netherlands). It can be assumed that also in the majority of the European and associated countries PFASs contamination in certain areas is a so far unidentified issue. In early 2017, a news alert has been published in *Science for Environment Policy* titled "Europe's rivers 'highly contaminated' with long-chain perfluoroalkyl acids", stating that all large European rivers are highly contaminated with perfluoroalkyl acids and further, that European environmental quality standards for PFOS are exceeded in all of them (EC, 2017). Recently, the PFOA replacement chemical GenX was detected at all downstream river sampling sites with the highest concentration (812 ng/L) at the first sampling location downstream from a fluorochemical production plant, which was 13 times higher than concentrations of sum perfluoroalkylcarboxylic acids and perfluoroalkanesulfonates (\sum PFCA+ \sum PFSA) (Gebbinck et al., 2017). Furthermore, there is a strong indication that PFASs are increasingly used in chemical products, processes and articles, and that they are more and more detected in various environmental matrices. The knowledge about their specific uses and therefore the sources of emissions as well as hazard and risk is poor for many of the substances in this group (KEMI, 2017). Especially very limited knowledge in the public domain on the structures, properties, uses and toxicological profiles of fluorinated alternatives is available. The levels of some fluorinated alternatives or their degradation products, such as perfluorobutane sulfonic acid (PFBS) or perfluorobutanoic acid (PFBA), have been shown to be rising in the environment and human tissues in recent years in Europe (Scheringer et al., 2014). Fluorotelomer market size estimations predict increasing demands globally as well as a rise in the consumption as shown by Global Market Insights (2016). The number of approved patents in the US with "perfluor" in the patent text has raised to more than 400 per month (Fischer, 2017).

One of the major societal concerns is the irreversibility of contamination, together with endocrine disrupting effects, carcinogenicity, toxicity to reproduction, effects on immune system and on lipid metabolism for a broad range of PFASs.

A briefing provided by Chemtrust points out that children are currently not sufficiently being protected from chemicals that can disrupt brain development; they list per- and polyfluorinated compounds as one of the chemicals substance groups of concern (Chemtrust, 2017a). Chemtrust also raises the issue of use of PFASs as food contact materials and refers to a report on the

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implementation of the Food Contact Materials Regulation of the European Parliament which states that action at EU level is needed to address the lack of EU specific measures and the gaps in risk assessment, traceability, compliance and control (EU parliament, 2016) and an assessment of the Joint Research Center on the regulatory and market situation of the non-harmonised food contact materials in the EU (Simoneau et al., 2016). Also, European consumer organisations call for action on fluorinated compounds in fast food packaging (BEUC, 2017).

Moreover the European Environmental Bureau (EEB) addressed concerns on exposure of humans to the big group of PFASs: “We would like HBM4EU to address in particular the lack of human exposure information on the substances of the group that are being used as alternatives to other substances of the group that are under regulatory activity, such a PFOS and PFOA” (EEB, 2017).

According to the EEA, PFASs contamination has the potential of a planetary boundary threat (Trier, 2017).

19.2 Categorisation of Substances

Based on the huge amount of available PFAS on the market and the knowledge gaps on identity, toxicity and uses (of the alternatives), the listing of chemicals in categories A-E is an attempt to categorise possibly relevant substances that contribute to the overall PFAS burden in humans. Several substances are listed in category A due to their restriction as PFOS- and PFOA-related substances, although limited or no HBM data are available. Efforts should be made to improve the methods to detect the broader spectrum of Category A substances. However, the priority for future HBM research should cover PFOS and PFOA alternatives with high production volume, wide dispersive use and identified or suspected hazardous properties which qualifies for SVHC identification. For substance selection the following issues were considered: availability of substance identity and literature, building blocks or alternative processing aid in polymer manufacturing, use as food contact material, alternatives to long-chain PFAS and degradation products/intermediates. Due to the variety of PFAS classes and structures it is clear that the list of substances in categories C-E is open ended and should regularly be updated.

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Table 19-1: Substances included in the substance group, listed according to availability of toxicology and human biomarker data, in category A, B, C,D, E substances (see general introduction)

Category	Abbrev./Acronym	Systematic name	CAS No.	Regulation
A	PFOA	Perfluorooctanoic acid (linear and branched isomers)	335-67-1	REACH Annex XVII restriction (Regulation (EU) 2017/1000) ⁴² , SVHC Candidate List (PBT, Repr.), CLH (Carc. 2, Repr. 1B, STOT RE 1, Acute Tox. 4, Eye Dam. 1), proposed for inclusion in the Stockholm Convention, Norman 2011, sufficient EU HBM data available
A	PFOS	Perfluorooctane sulphonate, Heptadecafluorooctane-1-sulphonic acid (linear and branched isomers)	1763-23-1	REACH Annex XVII restriction, CLH (Carc. 2, Repr. 1B, Lact., STOT RE 1, Acute Tox. 4, Aquatic Chron. 2), PIC regulation, POP Regulation (EG) No. 757/2010, Stockholm Convention, environmental legislation (Seveso, Directive 2012/18/EU; Regulation 649/2012 concerning export and import of hazardous chemicals), sufficient EU HBM data available
A	PFNA	perfluoro-n-nonanoic acid	375-95-1	Restriction proposal ⁴³ , CLH (Carc. 2, Lact., STOT RE 1, Repr. 1B, Acute Tox. 4, Eye Dam. 1), SVHC Candidate List (Repr., PBT), PACT list (CMR, PBT), Annex III Directive 2008/98/EC on waste, Norman 2011, EU HBM data available
A	PFDA	perfluoro-n-decanoic acid	335-76-2	Restriction proposal, SVHC Candidate list (PBT, Repr.), PACT list (PBT), Norman 2011, EU HBM data available
A	PFU(n)DA	perfluoro-n-undecanoic acid	2058-94-8	Restriction proposal, SVHC Candidate List (vPvB), self classification (Acute tox. 4, Skin irrit. 2, Eye irrit. 2, STOT SE 3), Norman 2011, EU HBM data available
A	PFDoDA	Perfluorodeconoic Acid	307-55-1	Restriction proposal, SVHC Candidate List (vPvB), self classification (Skin irrit. 2, Eye irrit. 2, STOT SE 3, Metal corr. 1, Skin corr. 1B, Eye dam. 1), Norman 2011, EU HBM data available
A	PFTTrDA	perfluoro-n-tridecanoic acid	72629-94-8	Restriction proposal, SVHC Candidate List (vPvB), self classification (Skin corr. 1B), EU HBM data available
A	PFTeDA	perfluoro-n-tetradecanoic acid	376-06-7	SVHC Candidate List (vPvB), Restriction proposal, Norman 2011, EU HBM data available
A	PFHxS	perfluoro-1-hexanesulfonate (linear and branched isomers)	355-46-4	PACT list, proposed for inclusion in the Stockholm Convention, Norman 2015, EU HBM data available, longest half-live in humans (8.5-30 years)

⁴² <http://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A32017R1000>

⁴³ https://echa.europa.eu/documents/10162/13641/rest_pfcas_axvreport_sps-013246-17_en.pdf/ab1c11b0-4ec9-4287-b9c5-32cb98607152

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Category	Abbrev./Acronym	Systematic name	CAS No.	Regulation
A	FOSA, PFOSA	Perfluorooctylsulfonamide; Perfluorooctanesulfonic acid amide or 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8- Heptadecafluoro-1- octanesulfonamide (IUPAC)	754-91-6	PFOS-related substance; POP Regulation (EG) No. 757/2010, OSH legislation (Council Directive 98/24/EC on protection of health and safety of workers), environmental legislation (Seveso, Directive 2012/18/EU), self classification (Acute tox. 3, Skin irrit. 2, Eye irrit. 2, STOT SE 3), Norman 2011, some (but not sufficient) EU HBM data available (e.g. Haug et al., 2009), other non-EU HBM data (e.g. Jin et al., 2016)
A	n-MeFOSA	N-methylperfluoro-1 octanesulphonamide 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8- Heptadecafluoro-N-methyl-1- octanesulfonamide (IUPAC)	31506-32-8	PFOS-related substance; POP Regulation (EG) No. 757/2010, PIC Regulation, Norman 2011, some (but not sufficient) EU HBM data available (e.g. Bartolomé et al., 2017), other non-EU HBM data (e.g. Jin et al., 2016; Yeung and Mabury, 2016)
A	N-Et-FOSAA, Et- PFOSA-AcOH, Et- FOSAA	N-Ethyl-perfluorooctanesulfonamido acetic acid; N-ethyl-perfluorooctane sulfonamidoacetate or N-ethyl-N- [(1,1,2,2,3,3,4,4,5,5,6,6,7, 7,8,8,8- heptadecafluoro octyl)sulfonyl]- glycine (IUPAC)	2991-50-6	PFOS-related substance, transformation product, POP Regulation (EG) No. 757/2010, its salts may be marketed under different trade names, may be marker of food or consumer exposures, Norman 2015, limited EU HBM data available (ELFE study – serum samples), other non-EU HBM data (e.g. Kato et al., 2014)
A	N-EtFOSA, SULFLURAMID	N-ethylperfluoro-1- octanesulphonamide or N-Ethyl- 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8- heptadecafluoro-1-octanesulfonamide (IUPAC)	4151-50-2	PFOS-related substance, POP Regulation (EG) No. 757/2010, PIC Regulation, environmental legislation (Seveso, Directive 2012/18/EU), self classification (Acute tox. 4), Norman 2011, investigated in human samples but not detected (Jin et al., 2016, Miyake et al., 2007, Yeung and Mabury, 2016), detected in indoor dust and air samples (Gebbinck et al., 2015)
A	N-EtFOSE	N-ethyl-perfluorooctane sulphonamidoethanol; N-Ethyl-N-(2- hydroxyethyl)perfluorooctanesulfona mide or N-Ethyl- 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8- heptadecafluoro-N-(2-hydroxyethyl)- 1-octanesulfonamide (IUPAC)	1691-99-2	PFOS-related substance; POP Regulation (EU) No. 850/2004 idgF, PIC Regulation, Norman 2011, detected in indoor dust and air samples (Gebbinck et al., 2015), quickly and extensively metabolised to PFOSA with an elimination half-life of 16-20 h, metabolites of N-EtFOSE were found in human samples (Thayer and Houlihan, 2002), HBM data scarcely available
A	N-MeFOSE	N-methyl perfluorooctanesulfonamidoethanol or 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8- heptadecafluoro-N-(2-hydroxyethyl)- N-methyloctane-1-sulfonamide (IUPAC)	24448-09-7	PFOS-related substance, POP Regulation (EU) No. 850/2004 idgF, PIC Regulation, detected in indoor dust and air samples (Gebbinck et al., 2015), no HBM data available at current knowledge

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A	8:2 diPAP	polyfluoroalkyl phosphoric acid diesters, Bis(3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-hepta-decafluorodecyl) hydrogen phosphate	678-41-1	PFOA-related substance, REACH Annex XVII restriction (Regulation (EU) 2017/1000), Norman 2015, Priority HBM List California, limited EU HBM data available (Yeung et al., 2013a, 2013b); other non-EU HBM data (e.g. Lee and Mabury, 2011; Yeung and Mabury, 2016)
A	6:2/8:2 diPAP	6:2/8:2 polyfluoroalkyl phosphoric acid diesters	943913-15-3	PFOA-related substance, REACH Annex XVII restriction (Regulation (EU) 2017/1000), Priority HBM List California, no HBM data available at current knowledge
A	8:2 monoPAP	8:2 polyfluoroalkyl phosphoric acid monoester	57678-03-2	PFOA-related substance, REACH Annex XVII restriction (Regulation (EU) 2017/1000), no HBM data available at current knowledge
B	TFA	Trifluoroacetic acid	76-05-1	Substance is used in formulation and re-packaging, at industrial sites and in manufacturing, recent HBM exposure data from Chinak
B	ADONA	Ammonium 4,8-dioxa-3H-perfluorononanoate (ammonium 2,2,3-trifluor-3-(1,1,2,2,3,3-hexafluoro-3-trifluormethoxypropoxy), propionate)	958445-44-8	Alternative to APFO, possible PPAR α antagonist, use in food contact material, according to EFSA no risk under specific conditions of use (EFSA, 2011 b), CoRAP (suspected PBT/vPvB, exposure of environment, wide dispersive use), highlighted by ECHA, limited EU HBM data available (e.g. Fromme et al., 2017)
B	PFBA	perfluoro-n-butanoic acid	375-22-4	REACH RMOA ⁴⁴ , Annex III (suspected P), OSH legislation (Council Directive 98/24/EC on protection of health and safety of workers), self classification (Skin corr. 1A, Eye dam. 1, STOT SE 3, Metal corr. 1), Norman 2015, highlighted by ECHA, levels rising in environment and human tissues (Scheringer et al., 2014), some (but not sufficient) EU HBM data available in plasma, serum, breast milk ⁴⁵
B	PFPeA	perfluoro-n-pentanoic acid	2706-90-3	REACH Annex III (suspected P, skin irritant), OSH legislation (Council Directive 98/24/EC on protection of health and safety of workers), self classification (Skin corr. 1B, Metal corr. 1, Eye dam. 1), Norman 2015, highlighted by ECHA, some (but not sufficient) EU HBM data available in plasma, serum, urine ⁴⁶
B	PFHxA	perfluoro-n-hexanoic acid	307-24-4	RMOA, REACH Annex III (suspected P, C), PACT list (PBT), OSH legislation (Council Directive 98/24/EC on protection of health and safety of workers), self classification (Skin corr. 1B, Metal corr. 1, Eye dam. 1, Acute tox. 4), Norman 2011, highlighted by ECHA, in Spain higher levels were found in liver samples (68-141 ng/g) (Perez et al., 2012), some (but not sufficient) EU HBM data available in plasma, serum, whole blood, breast milk, urine, liver ⁴⁷

⁴⁴ RMOA: Analysis of the most appropriate risk management option: <https://echa.europa.eu/de/rmoa>

⁴⁵ e.g. Antignac et al., 2013; Schröter-Kermani et al., 2013; Sochorová et al., 2017

⁴⁶ e.g. Hartmann et al., 2017; Schröter-Kermani et al., 2013; Sochorová et al., 2017

⁴⁷ e.g. Antignac et al., 2013; Ericson et al., 2007; Glynn et al., 2012; Hartmann et al., 2017; Perez et al., 2012; Schröter-Kermani et al., 2013; Sochorová et al., 2017

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B	PFHpA	Perfluoro-n-heptanoic acid	375-85-9	REACH Annex III (suspected B, P, C, acute tox via oral route, toxic), OSH legislation (Council Directive 98/24/EC on protection of health and safety of workers), self classification (Acute tox. 4, Skin corr. 1B, Metal corr. 1, Eye dam. 1), Norman 2011, highlighted by ECHA, some (but not sufficient) EU HBM data available in serum, plasma, whole blood, urine, breast milk ⁴⁸
B	PFBS	perfluoro-1-butanefulfonate	375-73-5	RMOA, PACT list, REACH Annex III (suspected P, R), OSH legislation (Council Directive 98/24/EC on protection of health and safety of workers, Council Directive 94/33/EC on the protection of young people at work), self classification (Acute Tox. 4, Skin corr. 1B, Metal corr. 1, Eye dam. 1), highlighted by ECHA, ground water contaminant, some (but not sufficient) EU HBM data available in serum, whole blood, breast milk ⁴⁹
B	PFHpS	perfluoro-heptanesulfonate	60270-55-5	REACH Annex III (suspected B, P, C, R, toxic, acute tox via oral route), self classification (Acute tox. 3, Eye irrit. 2, STOT SE 3), EU HBM data available
B	PFDS	perfluoro-1-decanesulfonate	335-77-3	REACH Annex III (suspected B, P, C, R, acute tox via oral route), some (but not sufficient) EU HBM data available in plasma, serum, breast milk, urine ⁵⁰
B	N-Me-PFOSA-AcOH, Me-FOSAA	N-Methyl-perfluorooctane sulfonamido acetic acid	2355-31-9	transformation product, may be marker of food or consumer exposures, limited EU HBM data available (ELFE study – serum samples), other non-EU HBM data (e.g. Kato et al., 2014)
B	6:2 FTSA, H4PFOS, THPFOS	3,3,4,4,5,5,6,6,7,7,8,8,8- tridecafluorooctanesulphonic acid, 6:2 fluorotelomer sulfonic acid	27619-97-2	REACH Annex III (suspected P, B, C, skin irritant), OSH legislation (Council Directive 98/24/EC on protection of health and safety of workers, Council Directive 94/33/EC on the protection of young people at work), Annex III Directive 2008/98/EC on waste, self classification (Skin corr. 1B, Eye dam. 1, Acute tox. 4, STOT RE 2), other non-EU HBM data (Yeung and Mabury, 2016)
B	8:2 FTSA	3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10- heptadecafluorodecanesulphonic acid, 8:2 fluorotelomer sulfonic acid	39108-34-4	REACH Annex III (suspected P, C, skin irritant), Priority HBM List California, other (non-EU) HBM data (Yeung and Mabury, 2016 – levels in all human blood samples below LOQ)
B	PFODA	Perfluorostearic acid; Perfluorooctadecanoic acid	16517-11-6	REACH Annex III (suspected C, P), priority HBM List California, limited EU HBM data available (Gebbinck et al., 2015)

⁴⁸ e.g. Antignac et al., 2013; Ericson et al., 2007; Glynn et al., 2012; Hartmann et al., 2017; Schröter-Kermani et al., 2013; Umweltbundesamt, 2013 (unpublished report)

⁴⁹ e.g. Ericson et al., 2007; Glynn et al., 2012; Umweltbundesamt, 2013 (unpublished report)

⁵⁰ e.g. Antignac et al., 2013; Hartmann et al., 2017; Haug et al., 2009; Schröter-Kermani et al., 2013; Umweltbundesamt, 2013 (unpublished report)

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Category	Abbrev./Acronym	Systematic name	CAS No.	Regulation
B	PfHxDA	Perfluoropalmitic acid, Perfluoro-n-hexadecanoic acid or 2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,13,13,14,14,15,15,16,16,16-Hentriacontafluorohexadecanoic acid (IUPAC)	67905-19-5	REACH Annex III (suspected P, B, C), Priority HBM List California, no HBM data available at current knowledge
C	4:2 FTSA	4:2 fluorotelomer sulfonic acid, 3,3,4,4,5,5,6,6,6-Nonafluoro-1-hexanesulfonic acid (IUPAC)	757124-72-4	Priority HBM List California, no EU HBM data available at current knowledge, other non-EU HBM data (Yeung and Mabury, 2016 – levels in all human blood samples below LOQ)
C	5:3 FTCA 7:3 FTCA	Fluorotelomer carboxylic acids 5:3 Fluorotelomer carboxylic acid 7:3 Fluorotelomer carboxylic acid	-	Fluorotelomer metabolites, Priority HBM List California, detected in blood samples of ski way technicians (Nilsson et al., 2013), HBM data scarcely available
C	6:2 FTUCA 8:2 FTUCA 10:2 FTUCA	Fluorotelomer unsaturated carboxylic acids 6:2 Fluorotelomer unsaturated carboxylic acid 8:2 Fluorotelomer unsaturated carboxylic acid 10:2 Fluorotelomer unsaturated carboxylic acid	70887-88-6 70887-84-2 70887-94-4	Fluorotelomer metabolites, Priority HBM List California, detected in blood samples of ski way technicians (Nilsson et al., 2013), HBM data scarcely available
C	PFECA (GenX)	Perfluoroether carboxylic acids for example: Ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate (GenX)	62037-80-3	CoRAP (suspected PBT/vPvB, exposure of environment), OSH legislation (Council Directive 98/24/EC on protection of health and safety of workers, Council Directive 94/33/EC on the protection of young people at work), Annex III Directive 2008/98/EC on waste, self classification (Acute tox. 4, Skin corr. 1C, Eye dam. 1, STOT SE 3, Skin corr. 1B), Norman 2015, highlighted by ECHA, no HBM data available at current knowledge
C	PFECA	perfluoro-1,1-ethylene glycol, terminated with chlorohexafluoropropoxy groups Perfluoro[[2-ethoxy-ethoxy]acetic acid], ammonium salt	908020-52-0	CoRAP (suspected PBT/vPvB, exposure of environment), resistant, not easily to metabolise, maybe bioaccumulative, expected increase in production and use, partially used in food contact materials, restriction on use according EFSA, no safety concern under the respective conditions identified (EFSA, 2011) no HBM data available at current knowledge

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Category	Abbrev./Acronym	Systematic name	CAS No.	Regulation
C	6:2 FTMAC	Fluorotelomer methacrylates e.g. 3,3,4,4,5,5,6,6,7,7,8,8,8- tridecafluorooctyl methacrylate	2144-53-8	CoRAP (potential endocrine disrupter, suspected PBT/vPvB, other hazard based concern, exposure of environment, wide dispersive use), PACT list, OSH legislation (Council Directive 98/24/EC on protection of health and safety of workers), self classification (STOT RE 2, STOT SE 3, Skin irrit. 2, Eye irrit. 2), fully registered substance 100-1.000 tonnes, suspected endocrine disrupter, used for polymer production, in human blood probably only metabolites detectable (FTOH, FTCA, FTUCAs), no HBM data available at current knowledge
C	6:2 FTAC 8:2 FTAC 10:2 FTAC	Fluorotelomer acrylates e.g. 6:2 Fluorotelomer acrylate (8:2 Fluorotelomer acrylate 10:2 Fluorotelomer acrylate)	17527-29-6 27905-45-9 17741-60-5	CAS# 17527-29-6: CoRAP (potential endocrine disrupter, suspected PBT/vPvB, other hazard based concern, exposure of environment, wide dispersive use), PACT list; CAS# 27905-45-9 and CAS# 17741-60-5: REACH Annex III (suspected P, C, respiratory sensitiser, skin irritant, skin sensitiser); FTAC is a PFOA-related compound; used for polymer production, Priority HBM List California, in human blood probably only metabolites detectable, no HBM data available at current knowledge
C	PfHxDA	Perfluoropalmitic acid, Perfluoro-n-hexadecanoic acid or 2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,13,13,14,14,15,15,16,16,16-Hentriacontafluorohexadecanoic acid (IUPAC)	67905-19-5	REACH Annex III (suspected P, B, C), Priority HBM List California, no HBM data available at current knowledge
C	C4/C4 PFPiA	Bis(nonafluorobutyl)phosphinic acid	52299-25-9	CoRAP (suspected PBT/vPvB, other hazard based concern, exposure of environment), no HBM data available at current knowledge
C	8:2 FTOH	8:2 fluorotelomer alcohol	678-39-7	CLH proposal (Repr 1B), OSH legislation (Council Directive 98/24/EC on protection of health and safety of workers), self classification (Skin irrit. 2, Eye irrit. 2, STOT SE 3), Norman 2011, no HBM data available at current knowledge
C	C6/C6 PFPiA	Bis(perfluorohexyl)phosphinic acid	40143-77-9	Limited HBM data available (Human sera , single and pooled donor sample 2009, US : <1 - 50.2 ng/Land <1 - 201.4 ng/L (median) Lee & Mabury (2011)
C	C6/C8 PFPiA	Bis(perfluorohexyloctyl)phosphinic acid	610800-34-5	Priority HBM List California, Limited HBM data available (Human serasingle and pooled donor sample 2009, US : <1 - 60.9 ng/L and <1 - 283.4 ng/L(median) Lee & Mabury (2011)
C	C8/C8 PFPiA	Bis(perfluorooctyl)phosphinic acid	40143-79-1	Limited HBM data available (Human sera , single and pooled donor sample 2009, US : <1 - 22.2 ng/Land <1 - 50.7 ng/L (median) Lee & Mabury (2011)
C	HFPO	hexafluoropropylene oxide	220182-27-4	Highlighted by ECHA, no HBM data available at current knowledge

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Category	Abbrev./Acronym	Systematic name	CAS No.	Regulation
	PFCHS	Cyclic PFSA e.g Cyclohexanesulfonic acid undecafluoro-, potassium salt Cyclohexanesulfonic acid, nonafluorobis(trifluoromethyl)-, potassium salt Perfluoro-4-ethylcyclohexane sulfonate	3107-18-4 68156-01-4 335-24-0	CAS# 3107-18-4, CAS# 68156-01-4, CAS# 335-24-0: REACH Annex III (suspected C, P) ; no HBM data available at current knowledge
	6:2/8:2 diPAP	6:2/8:2 polyfluoroalkyl phosphoric acid diesters	943913-15-3	PFOA-related substance, REACH Annex XVII restriction (Regulation (EU) 2017/1000), Priority HBM List California, no HBM data available at current knowledge
	8:2 monoPAP	8:2 polyfluoroalkyl phosphoric acid monoester	57678-03-2	PFOA-related substance, REACH Annex XVII restriction (Regulation (EU) 2017/1000), no HBM data available at current knowledge
	PFOPA	Perfluorooctylphosphonic acid, 2-(Perfluorohexyl)ethyl] phosphonic acid	252237-40-4	Sodium salt REACH registration (ECHA, 2017) High environmental exposure, Priority HBM List California, limited HBM data available, not detected in US human sera in Lee & Mabury (2011)
	Perfluorinated Siloxane	Trimethoxy(1H,1H,2H,2H-heptafluorodecyl)silane	83048-65-1	OSH legislation (Council Directive 98/24/EC on protection of health and safety of workers, Council Directive 94/33/EC on the protection of young people at work), Annex III Directive 2008/98/EC on waste, self classification (Skin irrit. 2, Eye irrit. 2, Skin corr. 1B), no HBM data available at current knowledge
	FL16.119	N-(2-methylcyclohexyl)-2,3,4,5,6-pentafluorobenzamide	1003050-32-5	more information on use and use levels needed EFSA (CEF panel, 2017) Other health hazard, no HBM data available at current knowledge
	6:2 FTCA 8:2 FTCA 10:2 FTCA	Fluorotelomer carboxylic acids: 6:2 Fluorotelomer carboxylic acid, 8:2 Fluorotelomer carboxylic acid 10:2 Fluorotelomer carboxylic acid	53826-12-3 27854-31-5 53826-13-4	Fluorotelomer metabolites, Priority HBM List California, no HBM data available at current knowledge

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Category	Abbrev./Acronym	Systematic name	CAS No.	Regulation
	PFECA	Perfluoro-1,2-propylene glycol and perfluoro-1,1-ethylene glycol, terminated with chlorohexafluoropropoxy groups Perfluoro[(2-ethoxy-ethoxy)acetic acid], ammonium salt	329238-24-6	resistent, not easily to metabolise, maybe bioaccumative, expected increase in production and use, partially used in food contact materials, restriction on use according EFSA, no safety concern under defined conditions (EFSA, 2010) no HBM data available at current knowledge
	FBSA	Perfluorobutane sulfonamide	30334-69-1	Alternative to PFOS, tranformation product, recently detected in biota (fish) (Cu et al., 2016)
	MeFBSE	N-Methyl perfluorobutane-sulfonamidoethanol	34454-97-2	REACH, registered substance, Intermediate; Surfactants; Repellents for porous hard surfaces; Tile grout additive, Registered 12 – 100 t/y
	6:2 PAP	6:2 polyfluoroalkyl phosphoric acid monoesters	57678-01-0	Priority HBM List California, no HBM data available at current knowledge
	6:2 diPAP	6:2 polyfluoroalkyl phosphoric acid diesters	57677-95-9	Priority HBM List California, no HBM data available at current knowledge
	PFHxPA	Perfluorohexylphosphonic acid	40143-76-8	high environmental exposure, Priority HBM List California, not detected in US human sera in Lee & Mabury (2011) limited HBM data available
	PFDPA	Perfluorodecylphosphonic acid	52299-26-0	Priority HBM List California, not detected in US human sera in Lee & Mabury (2011) limited HBM data available
	C8/C10 PFPiA	Bis(perfluorooctyldecyl)phosphinic acid	500776-81-8	no HBM data available at current knowledge
	Denum SH	Poly[oxy(1,1,2,2,3,3-hexafluoro-1,3-propanediyl)],a-(2-carboxy-1,1,2,2-tetrafluoroethyl)-w-(1,1,2,2,3,3,3-heptafluoropropoxy)-	120895-92-3	no HBM data available at current knowledge
	Krytox	Krytox-H	60164-51-4	no HBM data available at current knowledge
	Fomblin Z-DIAC,	Fomblin Z-DIAC, bis(pentafluorophenyl) ester	97462-40-1	no HBM data available at current knowledge
	-	C3; C15-C20 PFCA	-	no HBM data available at current knowledge
	-	C3, C15-C20 PFSA	-	no HBM data available at current knowledge
	TFEE-5	Polyfluoro-5,8,11,14-tetrakis(polyfluoroalkyl)-polyoxaalkane	-	CoRap, suspected PBT

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Category	Abbrev./Acronym	Systematic name	CAS No.	Regulation
	polymers: PTFE	Teflon: Polytetrafluoroethylene	9002-84-0	<p>More research on polymers</p> <p>Building blocks:</p> <p>CAS# 9002-84-0: REACH Annex III (suspected CMR);</p> <p>CAS# 24937-79-9: REACH Annex III (suspected P, M, hazardous to aquatic environment);</p> <p>CAS# 24981-14-4: -</p> <p>CAS# 116-14-3: PACT list (CMR);</p> <p>CAS# 116-15-4: CLH (Press. Gas, Acute Tox. 4, STOT SE 3), CoRAP (suspected CMR, high (aggregated) tonnage);</p> <p>production of toxic products if overheated; production of ultrafine particles by degradation; lung inflammation (PTFE), toxic monomers (PTFE); no HBM data available at current knowledge</p>
	PVDF	1,1 Difloroethene (PVDF)	24937-79-9	
	PVF	Polyvinyl fluorine (PVF)	24981-14-4	
	TFE	Tetrafluoroethylene (TFE)	116-14-3	
	HFP	Hexafluoropropylene (HFP)	116-15-4	

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19.3 Policy-related questions

1. What is the current exposure of the EU population to PFASs and do they exceed Guidance values (reference and HBM values), where available?
2. Are there differences in exposure of the EU population to regulated and non-regulated PFASs?
3. Has restriction of PFOS according to the POP Regulation led to a reduction in exposure, especially for children?
4. Is exposure driven by diet, consumer exposure, occupation or environmental contamination?
5. Which areas and environmental media in Europe are contaminated with PFASs?
6. How can this feed into an assessment of the TDI for PFOS and PFOA set by EFSA?
7. What is the impact of a pending 2016 ECHA decision to restrict the manufacturing, marketing and use of PFOA under REACH?
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?
9. Can differences in PFASs profiles be observed in different population groups and time periods?
10. What are the PFASs levels and health effects in vulnerable population groups?
11. How can mixture effects of environmental and human PFASs mixtures present to date be estimated?
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?
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?

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19.4 Research Activities to be undertaken

Table 19-2: Listing of research activities proposed to answer the policy questions summed up in 1.3

Policy question	Substance	Available knowledge related to policy question	Knowledge gaps / Activities needed to answer policy question
1	CAT A and B substances	Alternatives to PFOS (e.g. PfhXS, PFBS) are detected more frequently and in increasing concentrations	Proceed with collecting, combining, harmonising and comparing existing exposure data on PFASs WP 10
	PFOS and PFOA; mixture of PFOS, PFOA, PFNA, PfhXS	In 2018 EFSA has assessed the exposure and risk of PFOS and PFOA and concluded that a considerable part of the European population is exposed to levels which are exceeding the tolerable daily intakes. In 2020 the mixture of 4 PFAS has been assessed and an even lower risk level has been identified, concluding that especially children are exposed to higher levels as the tolerable weekly intakes.	Compare PFAS exposure values with the newly derived EFSA health guideline values, develop HBM4EU guidance values for PFOS and PFOA or PFAS mixtures Develop further the risk assessment of PFAS mixtures WP 5
	CAT A and B substances	The results of the aligned studies will deliver data of 6-12 PFAS in European teenagers. Various questions related to exposure and health effect will be addressed in WP10.	Based on the results a detailed data gap analysis should be performed, taking the respective human health related endpoints into consideration in order to address the question if health based guidelines are met or not. In order to specifically address health endpoints where currently insufficient data are available study protocols should include measurement of transaminases, cholesterol, immune parameters and thyroid hormones. Mixture effects should be considered, taking the similar mode of action for certain substances into consideration. Uncertainty regarding the total PFASs exposure has to be considered. WP 5, 8,9,10,15
2	CAT A and B probably C substances	To date PFOS and PFOA and PFNA and PfhXS represent half of the PFAS exposure, at least of those PFAS for which data are available	New targeted studies identifying a multitude of PFASs in human blood and urine including newly developed methods such as TOF or oxidisable fractions should be planned and performed, in order to be able to quantify also the so far unidentified compounds. Analyses should be further complemented by measurement of transaminases, cholesterol, immune parameters and thyroid hormones. Development of TOF and oxidisable fraction methods should be validated and harmonised in order to integrate them in planned and ongoing studies. This will be part of the next partnership, as it cannot be dealt within HBM4EU. WP 8,9, WP14 (effect biomarkers)

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Policy question	Substance	Available knowledge related to policy question	Knowledge gaps / Activities needed to answer policy question
3	PFOS	<p>The effectiveness evaluation under the UNEP Stockholm Convention concluded that for human matrices from Western Europe, Canada, Australia and Asia-Pacific countries levels seem gradually declining.</p> <p>It will most probably turn out that data on PFAS exposure in children is currently underrepresented; most studies performed within Europe are from adult populations with the exceptions of birth cohorts.</p>	<p>Exposure of children to PFASs should be investigated, complemented by measurement of transaminases, cholesterol, immune parameters and thyroid hormones.</p> <p>WP 8,9,10 and WP14 (effect biomarkers)</p>
4	Cat A and B substances	<p>Long chain PFASs exposure is presumed to be via diet; contribution of food additives and flavourings is so far not sufficiently investigated. Also knowledge on the exposure to short chain PFASs via diet (e.g. crops and vegetables) and drinking water is scarce.</p> <p>Further, information on exposure via various consumer product has to be considered.</p>	<p>All new studies performed within HBM4EU targeting PFASs should include a detailed questionnaires based on current knowledge on exposure pathways. Therefore a PFASs related questionnaire has been developed.</p> <p>WP 8,9 Link with dietary surveys if possible (WP11) External and internal modelling: WP12</p>
5	Cat A and B substances	<p>Currently there are several hot spots known in different countries (e.g. Germany, Sweden, Italy, Spain, Netherlands). It can be assumed that hot spots exist also in the majority of the European and associated countries.</p>	<p>HBM4EU intends to study the exposure of the general population, therefore hot spots and contamination issues were not specifically addressed. However, knowledge and information from partners involved in such activities (e.g. Sweden, Italy, Spain) will be taken into account and processed. E.g. the "Bioambient.es" project (IISCI, Spain) will be taken into account.</p> <p>http://democophes.blogs.isciii.es/2012/04/04/bioambient-es/ http://democophes.blogs.isciii.es/category/biomonitorizacion-espana/ Exposure modelling in relation with HBMdata (WP12)</p>
6	Cat A and B Substances	<p>The new EFSA opinion has been published in 2020. HBM4EU has taken part in the public consultation.</p>	<p>The detailed EFSA assessments (2018, 2020) will be used within HBM4EU for defining data gaps and refining research questions. Based on previous information exchange and discussion among HBM4EU partners it is clear that there are several questions on human health that have to date not been sufficiently addressed due to relatively small size of many previous studies. Combining several comparable studies will allow for more robust assessment of health outcomes in terms or broader exposure range and examination of rare health outcomes which individual studies have been underpowered to address (including low birth weight, pregnancy complications).</p> <p>WP 5, 10, 13</p>

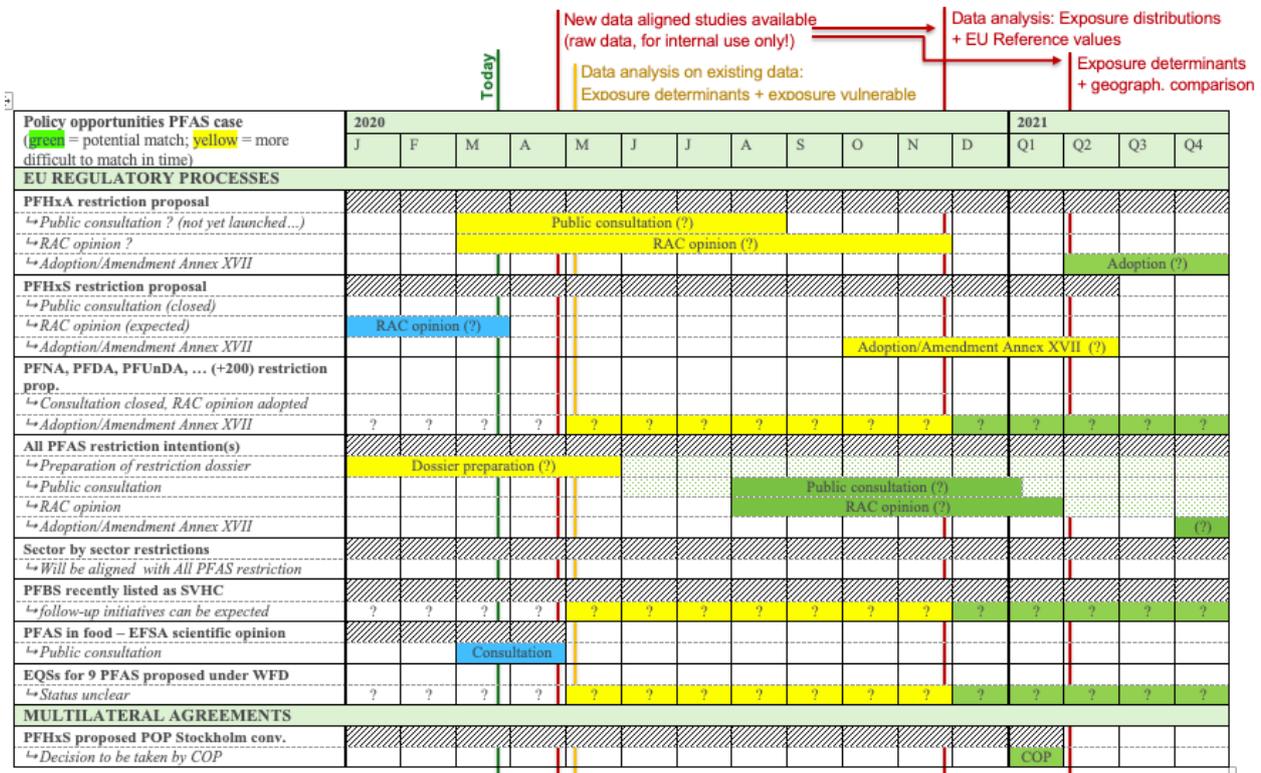
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Policy question	Substance	Available knowledge related to policy question	Knowledge gaps / Activities needed to answer policy question
7	PFOA and related substances	The restriction is expected to lead to declining levels of PFOA	The identification, assessment and monitoring of alternatives is of importance. WP 4, 5 WP 10 time trend data analysis
8	Cat A Substances	According to experts in different fields it is anticipated to eliminate legacy PFASs from waste streams.	It is not clear whether this question can be tackled within HBM4EU; Research on the life cycle of products may identify potential exposure routes. [Studies near landfills could clarify if PFASs exposure occurs.] WP 7,8,9
9	Cat A, B, C substances	Differences in exposure levels vary over the time, this has been documented in the recent EFSA assessment, there is also knowledge on other substances and from other studies (e.g. Yeung et al, 2008, 2013 a, b, 2016, Daun 2020)	To identify differences in the exposure levels of unregulated and regulated Cat. A substances (and Cat B and C substances if data are available) between countries and time periods, and to identify the main reasons for differences in exposure.*Population groups: living in different areas and divided by sex and gender. WP 10, 12
10	Cat A , B and C substances	As PFASs exposure pattern are changing current exposure of vulnerable populations needs to be investigated.	Current exposure levels in vulnerable populations need to be investigated, preferable with methods, which allow identifying Cat A , B and C substances as well as the total PFASs burden. The total PFAS burden will most probably assessed within the future EU HBM partnership, not within HBM4EU. *Vulnerable population: children (high half lives of PFAS) and those affected by health effects linked to the potential PFAS exposure. WP 8,9 WP13 exposure effect studies
10	Cat A substances	EFSA has assessed the risk of human exposure to the 4 most abundant PFAS and identified several endpoints of concern, but also additional concerns, which could not be verified within this assessment.	Study how PFAS affect critical endpoints in humans such as liver and thyroid, developmental toxicity, immunotoxicity and non carcinogenic toxicogenicity. Prenatal exposure is suspected to cause reduced birth weight and or small for gestational age, suggesting unborn as vulnerable exposure group. WP 13
11	Mixture of substances Identification of Cat E substances	EFSA has assessed the risk of human exposure to the 4 most abundant PFAS, there are however remaining uncertainties.	To address questions related to mixture effects (due to similar mode of action and potential over-additive effects of combined exposures): e.g. peroxisome proliferation, mitochondrial toxicity, cytotoxicity, and transcriptome profiles of key metabolic pathways of the liver, immunotoxicity reproductive, developmental and carcinogenic effects and also address multipollutant (PFASs) exposure in relation to adverse health effects in epidemiological studies WP 5, 13, 14, 15

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Policy question	Substance	Available knowledge related to policy question	Knowledge gaps / Activities needed to answer policy question
12	Cat D and E substances	What compounds 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 studies?	Identification of compounds to be prioritised for further information on exposure and/or toxicity to be measured in HBM studies WP 4,5 Identify lead chemicals in mixtures of PFAs WP14 and WP15 Design new studies that measure these exposure biomarkers including the total and extractable organic fluorine, WP8 within the future partnership
14	PFOA/PFOS	There is evidence of wide variability in half life, with gender, renal function and genetics shown to explain some of the variation and HBM levels.	Taking into consideration the differences in toxicokinetics of linear and branched isomers, fuller characterisation of role of gender, existing disease use of medicines and other causes affecting measured HBM in serum This will not explicitly be part of HBM4EU, some questions might be addressed within WP10.

The table below depicts the policy alignment for PFAS in the frame of HBM4EU (additional Deliverable WP5: 'Timelines of Opportunity', submitted to the coordinator)



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20 Prioritised substance group: Pesticides

Pyrethroids (group), chlorpyrifos, dimethoate, glyphosate (including the co-formulant POE-tallow amine) and fipronil (D.4.5)

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20.1 Background Information

20.1.1 Hazardous properties

Regulatory hazard classifications of the substances are shown in Table 20. The purpose of this section is to identify knowledge gaps and to pinpoint research areas where epidemiological studies have raised concern for potential adverse health effects in humans at population-level exposures and where more HBM data are needed to further evaluate safe exposure levels (i.e., to answer the policy-related questions). This is especially important for health outcomes for which animal models might not be sufficiently sensitive, e.g. for developmental neurotoxicity (Fritsche et al. 2018) or for which valid animal models do not exist as for example for childhood leukemia (EFSA Panel on Plant Protection Products and their residues (PPR) et al. 2017).

20.1.1.1 Pyrethroids

Pyrethroids compose one of the major classes of insecticides in the EU and worldwide. They are synthetic analogs of pyrethrins naturally present in the Chrysanthemum flower but, compared to the pyrethrins, they are less susceptible to hydrolysis and photodegradation and therefore more stable in the environment. They are highly toxic to insects, but also fish and cats are particularly sensitive to pyrethroids toxicity. Pyrethroids are chiral compounds and the formulations consist of multiple stereoisomers which often have different toxic potencies and toxicokinetic. Based on structural differences and on signs of acute toxicity in rodents, pyrethroids are divided into type I and type II. Type 1 (e.g. permethrin and allethrin) comprise a wide structural variety of compounds lacking a cyano moiety at the alpha-position and elicit an intoxication syndrome that includes general tremor, convulsive twitching, hypersensitivity and aggression, designated the T (tremor) syndrome. Distinctively, type II pyrethroids (e.g., cypermethrin and deltamethrin) contain the the α -cyano-3- phenoxybenzyl moiety and cause an intoxication syndrome that includes salivation and progressive writhing convulsions (choreoathetosis) designated the CS syndrome. However, some pyrethroids exhibit intermediate signs of intoxication that contain elements of both the T and CS syndromes. (Soderlund 2012). The main mechanism of action for both types of pyrethroids is axonal sodium channel depolarisation causing repetitive nerve impulses both in insects and non-target organisms including mammals. However, each type of pyrethroids exhibit secondary targets, including voltage-gated calcium and gamma-aminobutyric acid (GABA)-gated chloride channels, also involved in their acute neurotoxic actions (Soderlund 2012). Increased sensitivity to acute pyrethroid toxicity during early development (the neonatal period), as seen in animal studies, was suggested to be due to lower metabolic capacity and expression of a more sensitive form of the voltage-gated sodium channel (Meacham et al. 2008).

Compared with other major classes of insecticides, like organophosphates and carbamates, pyrethroids have lower acute toxicity in mammals. However, potential human health effects of pyrethroids at low environmental and dietary exposure levels have only been addressed in few epidemiological studies, despite pyrethroids were introduced for the control of insect pest more

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than three decades ago. Since pyrethroids are known neurotoxicants and some have endocrine disrupting properties *in vitro* (Saillenfait et al. 2016a), pyrethroids have the potential to interfere with neurodevelopment (Abreu-Villaca and Levin 2017; Bjorling-Poulsen et al. 2008) and to disturb neuroendocrine axes and reproductive development (Koureas et al. 2012; Saillenfait et al. 2015), especially if exposure occurs in vulnerable developmental periods during childhood and foetal life.

Developmental neurotoxicity (DNT)

In research studies using animal models, developmental exposures to pyrethroids has been related to a wide range of behavioural, neurochemical and molecular effects including altered brain vascular formation, increased blood-brain barrier permeability, decreased monoamine levels and neocortical and hippocampal thickness, alterations in cholinergic muscarinic, dopaminergic and noradrenergic systems, delayed physical and motor development, decreased locomotor activity, impaired motor coordination, and deficient learning and memory, reviewed by (Abreu-Villaca and Levin 2017). In a recent study, offspring of mice orally exposed to the pyrethroid deltamethrin during gestation and lactation showed several ADHD-like features, including hyperactivity, impulse-like behaviours, and deficits in working memory and attention. Elevated dopamine transporter levels, lower synaptic dopamine, and increased D1 dopamine receptor levels accompanied the behavioural effects (Richardson et al. 2015). Although the pathophysiology of ADHD in humans is poorly understood, disruption of dopaminergic, noradrenergic, and serotonergic neurotransmission has been suggested to be central mechanisms (Thapar and Cooper 2016). Thus, these findings indicate that pyrethroids might interfere with neurobehavioral development in humans. Accordingly, some recent epidemiological studies reported associations between exposure to pyrethroids during pregnancy (evaluated by biomonitoring of maternal urinary pyrethroid metabolites) and lower cognitive scores at three months of age (Fluegge et al. 2016), at 12 months of age (Xue et al. 2013), and at 24, but not at 36, months of age (Watkins et al. 2016). One study, found no associations with child cognition at 12 months of age but with lower Social-Emotional scores on the Bayley Scales of Infant Development (Eskenazi et al. 2018). These findings were also supported by a study from New York City in which detectable levels of pyrethroid metabolites in maternal urine were associated with a variety of behavioural functioning deficits among children measured at four, six, and seven to nine years of age by the Behavioural Assessment System for Children and the Behaviour Rating Inventory of Executive Function (Furlong et al. 2017). Likewise, maternal pyrethroid exposure was not associated with cognitive development among the children at age 6 years in the French PELAGIE Cohort (Viel et al. 2015) but with internalising difficulties assessed by the Strengths and Difficulties Questionnaire (Viel et al. 2017), despite the exposure level in this cohort was lower than reported from other cohort studies, i.e., the common pyrethroid metabolite 3-PBA was only detectable in urine samples from 30 % of the women compared to 80-90 % in other studies (McKelvey et al. 2013; Wielgomas et al. 2013).

Since growth and functional development of the human brain continues during childhood, it is assumed that the postnatal period is also vulnerable to neurotoxic exposures (Grandjean and Landrigan 2006).

Accordingly, childhood pyrethroid exposure (child urinary concentrations of pyrethroid metabolites) has been associated with impaired cognitive functions, especially verbal and memory functions (Viel et al. 2015) and increased risk of behavioural problems (Oulhote and Bouchard 2013; Viel et al. 2017) including attention-deficit hyperactive disorder (ADHD) (Wagner-Schuman et al. 2015) even at very low urinary metabolite concentrations as reported from the French PELAGIE-cohort (Viel et al. 2015; Viel et al. 2017). Bifenthrin and alpha-cypermethrin are classified as STOT RE 1 – H372 and SOT RE2 – H373 (Table 3) for effects on the nervous system but they are not classified as developmental neurotoxicants.

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Endocrine disrupting properties and carcinogenicity

Besides neurotoxic properties, some pyrethroids, or their metabolites, have been reported to possess endocrine disrupting properties *in vitro* (Brander et al. 2012; Saillenfait et al. 2016a) and several pyrethroids (permethrin, acrinathrin, bifenthrin, cyfluthrin, cypermethrin, deltamethrin, esfenvalerate, etofenprox, lambda-cyhalothrin, tau-fluvalinate and tefluthrin) were categorised as potential endocrine disruptors (EDs) in an impact assessment report of JRC (EC 2016) (see Table 1). Accordingly, most of the pyrethroids approved for use in the EU are included in the TEDX list.

In an EU assessment report from 2014, (Reg (EU) No 528/2012) permethrin was reported to cause histopathological changes in the adrenals and increased liver weight in dogs of both sexes (NOAEL: 5 mg/kg bw/d). In experimental studies, the pyrethroid fenvalerate caused increased gonadotropins and a decline in testosterone in male rats (Mani et al. 2002). Perinatal exposure to cypermethrin, disturbed sexual maturation and later reproductive function in rat male offspring (Singh et al. 2017). Exposure to deltamethrin throughout gestation and lactation caused shorter ano-genital distance (AGD) in male offspring (Kilian et al. 2007) indicating insufficient androgen action, whereas no effects on AGD or on expression of genes involved in testicular steroidogenesis was observed when the exposure period was restricted to the period of sexual differentiation between gestational day 13 and 19 (Saillenfait et al. 2016b).

Several recent epidemiological studies have raised concerns about potentially adverse effects on sperm quality and sperm DNA, reproductive hormones, and pregnancy outcome (Saillenfait et al. 2015). Hence, population representative urinary concentrations of pyrethroid metabolites, have been associated with reduced semen quality (Meeker et al. 2008), higher serum concentrations of FSH and LH, and lower inhibin B, and testosterone (Meeker et al. 2009) and sperm aneuploidy (Radwan et al. 2015). Among Chinese women, urinary concentrations of pyrethroid metabolites were significantly associated with increased risk of primary ovarian function (POI) (Li et al. 2018).

Only very few human studies have addressed other health outcomes and potential associations with e.g., carcinogenicity, immune system function, and metabolic disturbances are unclear (Saillenfait et al. 2015; Xiao et al. 2017). Residential exposure to insecticides after indoor use was associated with increased risk of childhood leukaemia (Chen et al. 2015; Ntzani et al. 2013) as also reported in meta-analyses (Bailey et al. 2015; Chen et al. 2015). In most studies it was not possible to pinpoint specific pesticides but pyrethroids constitute the major group of insecticides used for indoor pest control and a study from Shanghai reported elevated risk of childhood leukaemia associated with urinary levels of pyrethroid metabolites in the children (Ding et al. 2012).

At present, none of the pyrethroids at the EU market is classified as reproductive toxicants (H360-H361d) but etofenprox is classified as a lactational hazard (H362) (Table 3). Bifenthrin is classified as “suspected of causing cancer” (Carc. 2 – H351).

A few pyrethroids (permethrin, fenvalerate and deltamethrin) were reviewed by IARC in 1991 (Volume 53) and assigned to Group 3 (not classifiable as to its carcinogenicity to humans). Permethrin is currently listed as a high priority compound for assessment by IARC and was classified as “likely to be carcinogenic to humans” after oral exposure by the US EPA in 2009 (EPA 2009). Permethrin is also listed on the Annex III inventory as it meets the mutagenicity criteria of Annex III to the REACH regulation. Furthermore, genotoxic properties for different pyrethroids have been indicated in experimental studies (Muranli 2013; Ramos-Chavez et al. 2015; Vardavas et al. 2016).

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Also, immunotoxic properties have been indicated in some experimental studies for bifenthrin (Wang et al. 2017) and deltamethrin (Kumar et al. 2015). Permethrin, bifenthrin and esfenvalerate are classified as skin sensitizers (Table 3).

Piperonyl butoxide (PBO) - pyrethroid co-formulant

Pyrethroids are often applied in combination with piperonyl butoxide (PBO), a cytochrome P450 inhibitor causing decreased breakdown of the pyrethroids. In the insects. PBO is also a known inhibitor of human cytochrome P450. Health effects related to use of pyrethroid-containing products may thus be due to combined or synergistic action of the pyrethroid and the synergist PBO. Accordingly, PBO, but not permethrin, measured in maternal hair samples during pregnancy was associated with impaired neurodevelopment at 36 months of age (Horton et al. 2011) and cough in the children at age 5-6 years (Liu et al. 2012). However, whether PBO is a causal factor or rather a proxy for the total pyrethroid exposure cannot be ruled out from the cited study.

PBO is approved as a BP (T18) in the EU (Reg (EU) 2016/2288) (see EU [assessment report](http://dissemination.echa.europa.eu/Biocides/ActiveSubstances/1344-18/1344-18_Assessment_Report.pdf): http://dissemination.echa.europa.eu/Biocides/ActiveSubstances/1344-18/1344-18_Assessment_Report.pdf) with proposed C&L as Carc.2; H351 (increased incidence of hepatocellular adenomas and carcinomas in mouse), STOT SE 3; H335 (respiratory tract irritation), EUH066. The long and medium term AELs and ADI are equal to 0,2 mg/kg bw/d and the AEL short term is 1,0 mg/kg bw/d. The substance is included in the CoRAP for Substance Evaluation, scheduled to start in 2019 by Sweden. According to the CoRAP justification document, it will be evaluated for ED and PBT properties (<https://echa.europa.eu/substance-information/-/substanceinfo/100.000.070>)

20.1.1.2 Chlorpyrifos and dimethoate (organophosphates)

Developmental neurotoxicity

Chlorpyrifos and dimethoate are organophosphate (OP) insecticides and both compounds are suspected developmental neurotoxicants and endocrine disruptors. Generally, OPs irreversibly inhibit acetylcholinesterase (AChE), the enzyme that catalyses the breakdown of acetylcholine (ACh) to acetate and choline in synaptic clefts in both insects and off-target organisms' nervous system. In humans and other mammals, when AChE inhibition exceeds 70–75%, acute poisoning results in a severe “cholinergic syndrome”, in which accumulation of acetylcholin leads to peripheral signs such as increased sweating and salivation, bronchoconstriction, miosis, increased gastrointestinal motility and tremors; and central nervous system effects such as dizziness, mental confusion, and eventually, convulsions and death (Krieger 2001).

Chlorpyrifos is metabolised to the more toxic intermediate chlorpyrifos-oxon (bioactivation), which is a strong inhibitor of AChE in brain, peripheral tissue, and serum and red blood cells. Besides, AChE inhibition, OPs have been shown in experimental studies to induce a variety of neurotoxic effects, particularly after developmental exposure, even at doses devoid of systemic toxicity.

Hence, developmental OP exposure has been associated with altered function of numerous proteins other than AChE and these additional mechanisms are suggested to be involved in the developmental neurotoxicity of these substances, although the exact mechanisms is not understood (for review see Abreu-Villaca and Levin (2017)). Exposure to chlorpyrifos during developmental has been reported to disrupt neuronal cell replication and differentiation through a variety of cellular mechanisms, culminating in loss of neurons, “mis-wiring” of brain circuits and deficiencies in synaptic function (Slotkin and Seidler 2005, 2009; Slotkin et al. 2012).

Thus, disturbance of brain development is the main health concern related to OP exposure in general and to chlorpyrifos in particular. Several reviews of neurodevelopmental effects of OP in

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humans have been conducted and most of them conclude that exposure during pregnancy, at levels found among groups of the general population, may have negative effects on children's neurodevelopment (Gonzalez-Alzaga et al. 2014; Munoz-Quezada et al. 2013; Ross et al. 2013).

Most of the human studies have been carried out in the US and have focused on assessing brain functions in children in relation to prenatal organophosphate exposure. In a longitudinal birth cohort study among farmworkers in California (the CHAMACOS cohort), maternal urinary concentrations of organophosphate metabolites in pregnancy were associated with abnormal reflexes in neonates (Young et al. 2005), adverse mental development at two years of age (Eskenazi et al. 2007), attention problems at three and a half and five years (Marks et al. 2010), and poorer intellectual development at seven years (Bouchard et al. 2011), and higher parent and teacher reported autism spectrum disorder scores at age 7 to 14 years (Sagiv et al. 2018). In accordance with this, a birth cohort study from New York reported impaired cognitive development at the ages 12 and 24 months and six to nine years related to maternal urine concentrations of organophosphates in pregnancy (Engel et al. 2011). However, some recent studies, based on cohorts of pregnant women recruited from the general population and without occupational or extensive residential exposure, did not find indication of impaired neurodevelopment in the children at 1-5 years of age (Donauer et al. 2016) or 6 years of age (Cartier et al. 2016) associated with maternal urinary concentrations of organophosphate in pregnancy. The later study, from the French PELAGIE cohort reported two to six times lower OP metabolite concentrations for pregnant women, than reported from other European studies as well as in studies from the US and Canada (Marks et al. 2010; Spaan et al. 2015; Yolton et al. 2013) (Annex 1, Table 2).

Regarding childhood exposure level, five-year-old children from the CHAMACOS cohort had higher risk scores for development of attention deficit hyperactive disorder (ADHD) if their urine concentration of organophosphate metabolites was elevated (Marks et al. 2010). Based on cross-sectional data from NHANES in the US, the risk of developing ADHD increased by 55 % for a ten-fold increase in urinary concentration of organophosphate metabolites in children between eight and 15 years (Bouchard et al. 2010).

Chlorpyrifos was until 2020 the most used OP in the EU (non-renewal of authorisation from February 2020) and worldwide and it is also the best studied OP in both animal models and in vitro studies. There is evidence for developmental neurotoxicity of chlorpyrifos both from experimental and epidemiological studies (Abreu-Villaca and Levin 2017).

The strongest evidence for neurodevelopmental effects in humans comes from a study performed at the Columbia Children's Center for Environmental Health (CCCEH) at Columbia University in New York. This inner-city birth cohort study was initiated before chlorpyrifos was banned for residential use in 2000 in the US.

The concentration of chlorpyrifos in umbilical cord blood was significantly associated with delayed psychomotor and mental development in children in the first three years of life (Rauh et al. 2006), poorer working memory and full-scale IQ at seven years of age (Rauh et al. 2011), structural changes, including decreased cortical thickness, in the brain of the children at school age (Rauh et al. 2012), and mild to moderate tremor in the arms at 11 years of age (Rauh et al. 2015). Based on these and other birth cohort studies, chlorpyrifos has been categorised as a human developmental neurotoxicant (Grandjean and Landrigan 2014), but these results were not included when setting the ADI value for chlorpyrifos in the 2014 EU regulatory risk assessment (European Food Safety Authority 2014b). The ADI was reduced from 0.01 to 0.001 mg/kg bw per day based on NOAELs of 0.1 mg/kg bw per day obtained from 2-year rat and dog studies with RBC AChE inhibition as the most sensitive end point (European Food Safety Authority 2014a). However, a risk assessment of chlorpyrifos from the US EPA in 2016 (Britton 2016) concluded that the effects observed in the

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CCCEH, with supporting results from the other 2 U.S. cohort studies and the seven additional epidemiological studies reviewed in 2015, provides sufficient evidence that there are neurodevelopmental effects occurring at chlorpyrifos exposure levels below those causing a 10% inhibition of AChE activity in red blood cells (RBC), which is currently used as point of departure for regulatory actions. Chlorpyrifos was re-evaluated by EFSA in 2019. The overall conclusion of the evaluation in relation to impacts on human health, was that:

“The information available indicates that the approval criteria as set out in Article 4(1) to (3) of Regulation (EC) No 1107/2009 are not satisfied as concerns were identified with regards to:

- ▶ The genotoxic potential of chlorpyrifos, which can not be ruled out based on the information available - positive findings were found in an in vitro chromosome aberration study and two in vitro unscheduled DNA synthesis assays; in vivo positive findings were found in open literature on chromosome aberration and on DNA damage caused through oxidative stress or by topoisomerase II inhibition which is considered a molecular initiating event for infant leukaemia. Consequently, health-based reference values cannot be established for chlorpyrifos and the dietary and non-dietary risk assessments cannot be conducted.
- ▶ Developmental neurotoxicity (DNT) - effects were observed in the available study on developmental neurotoxicity in rats (adverse effects were seen at the lowest dose tested in rats and a no observed adverse effects level ‘NOAEL’ could not be established) and epidemiological evidence exists showing an association between exposure to chlorpyrifos and/or chlorpyrifos-methyl¹³ during development and adverse neurodevelopmental outcomes in children.
- ▶ Based on the evidence for DNT, experts during the peer review suggested that classification of chlorpyrifos as toxic for reproduction, category 1B, H360D ‘May damage the unborn child’, in accordance with the criteria set out in Commission Regulation (EC) No 1272/2008¹⁴ would be appropriate.”

Accordingly, approval of chlorpyrifos was not renewed. A very similar conclusion was drawn for chlorpyrifos-methyl, and the authorisation for both substances in the EU was withdrawn by 16 February 2020. However, both compounds are still used outside the EU and therefore residues in food is still a source of exposure.

Endocrine disrupting properties

Both chlorpyrifos and dimethoate are suspected endocrine disrupting substances (EC 2016) (Table 1) and included in the TEDX list. Chlorpyrifos has been reported to disrupt thyroid function in animal studies. In rat studies, a reduction in brain T₄ levels was seen following prenatal chlorpyrifos exposure whereas postnatal exposure caused a transient elevation in young adulthood (Slotkin et al. 2013). Mice exposed to chlorpyrifos postnatally, at doses that did not cause cholinesterase inhibition, showed a small, but significant reduction in serum concentrations of triiodothyronine and thyroxine (T₄). The effect was selective for males and was associated with cellular abnormalities in the thyroid gland (De Angelis et al. 2009). Given the importance of thyroid hormones for brain development (Korevaar et al. 2016) disturbance of brain thyroid hormone levels and function may contribute to neurobehavioral deficits associated with chlorpyrifos exposure. In rats, perinatal low-dose exposure to chlorpyrifos caused disrupted glucose and lipid homeostasis, and excess weight gain in adulthood (Lassiter and Brimijoin 2008; Slotkin 2011). Similar effects have been reported for other OPs and occupational exposure to OPs has been associated with increased risk of obesity and type 2 diabetes (Evangelou et al. 2016; Xiao et al. 2017). Whether exposure levels seen in the general population can disturb glucose and/or lipid metabolism is not known at present.

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Both chlorpyrifos and dimethoate decreased the expression of the steroidogenic acute regulatory (StAR) gene and thereby inhibit steroidogenesis in Leydig cell assays (Viswanath et al. 2010; Walsh et al. 2000b). Among male floriculture workers, urinary concentrations of organophosphate metabolites were associated with increased serum concentrations of FSH and prolactin and with decreased serum testosterone and inhibin B (Aguilar-Garduno et al. 2013). In rats, chlorpyrifos at low oral doses (0.01 mg/kg/day) for 100 days increased the number of ducts and alveolar structures in the mammary gland and the incidence of benign proliferative lesions in the mammary gland of these animals. In addition, circulating steroid hormones and gonadotrophins levels were reduced (Ventura et al. 2016).

Carcinogenicity and immunotoxicity

Only very few human studies have addressed other health outcomes related to chlorpyrifos or to general OP exposure and potential associations with e.g., carcinogenicity, reproductive function, and immune system function are not clear. Neither chlorpyrifos nor dimethoate are classified as reproductive toxicants or carcinogenic but in the latest EFSA risk assessment, no toxicological reference values were established for dimethoate due to genotoxicity concerns because of mutagenic effects in bacterial and mammalian cells (European Food Safety Authority (EFSA) 2018). Some epidemiological studies have associated chlorpyrifos with cancer risk, e.g. lung, rectal, and breast cancer and increased risk of Non-Hodgkin Lymphoma (Alavanja et al. 2004; Engel et al. 2017; Lee et al. 2004; Lee et al. 2007; Waddell et al. 2001).

20.1.1.3 Glyphosate and POEA

The herbicidal action of glyphosate derives from its inhibition of a key plant enzyme, 5-enolpyruvylshikimate-3-phosphate synthase, which is involved in the synthesis of aromatic amino acids. Since this enzyme is not present in vertebrates, it has long been assumed that glyphosate would not affect non-target species, including humans.

In plants and the environment, glyphosate is mainly degraded to aminomethylphosphonic acid (AMPA). In the EFSA risk assessment of glyphosate, it was concluded that AMPA presents a similar toxicological profile to glyphosate and the health guidance values (e.g., ADI) of the latter apply to its metabolite AMPA. No toxicological data were provided on *N*-acetyl-glyphosate (NAG) and *N*-acetyl-AMPA which were identified as relevant compounds in plant/livestock residues where glyphosate tolerant genetically modified (GM) plant varieties are eaten by humans or farm animals. The need for information on this was identified as a data gap (European Food Safety Authority 2015a).

Carcinogenicity and immunotoxicity

In 2015 IARC classified glyphosate as probably carcinogenic to humans (Group 2A) (Guyton et al. 2015), a classification that considerably triggered the debate over health risks of this substance. A 2016 EFSA review of the carcinogenic potential of glyphosate concluded that glyphosate is unlikely to pose a carcinogenic hazard to humans and the evidence does not support classification with regard to its carcinogenic potential according to Regulation (EC) No 1272/2008 (European Food Safety Authority 2015a). In 2017 ECHA – RAC (Risk Assessment Committee) assessed glyphosate's hazardousness and concluded that the scientific evidence available at the moment warrants the following classifications for glyphosate according to the CLP Regulation: Eye Damage 1; H318 (Causes serious eye damage), Aquatic Chronic 2; H411 (Toxic to aquatic life with long lasting effects). RAC concluded that the available scientific evidence did not meet the criteria in the CLP Regulation to classify glyphosate for specific target organ toxicity, or as a carcinogen, as a mutagen or for reproductive toxicity (ECHA, 2017). The Joint FAO/WHO Meeting on Pesticide Residues (JMPR) concluded in 2016 that glyphosate is unlikely to pose a carcinogenic risk to

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humans from exposure through the diet (JMPR 2017). The US-EPA has also classified glyphosate as “Not likely to be carcinogenic to humans” while the US state of California recently decided to list glyphosate as cancer causing (July 2017). Likewise, the Danish Working Environment Authority (WEA) has listed glyphosate as a carcinogen in 2015.

Potential explanations for the controversy in evaluation of glyphosate has subsequently been discussed (Clausing et al. 2018; Portier et al. 2016; Tarazona et al. 2017; Vandenberg et al. 2017; Williams et al. 2016) and might partly be related to differences in toxicity between glyphosate (alone) and GBH-formulations. The IARC classification was based on evaluation of both GBHs (including co-formulants as POEA, see below) and glyphosate alone. The epidemiological evidence includes two meta-analyses, both of which found significant increased risk of non-Hodgkin’s lymphoma (NHL) associated with occupational exposure to GBH (Chang and Delzell 2016; Schinasi and Leon 2014).

The issue of potential higher toxicity related to GBH-formulations than to “pure” glyphosate is not specific to genotoxicity and/or carcinogenicity and has also been reported for other endpoints in experimental studies, also at doses below regulatory limits for glyphosate (i.e., NOAEL of 50 mg/kg bw/day) (Mesnage et al. 2015). However, at a regulatory level, glyphosate is tested alone for chronic toxicity in animal studies and the data are used for setting ADI and other regulatory norms for glyphosate alone, even though it is never used in this form but only as part of a mixture with adjuvants in the commercial formulations. Accordingly, EFSA has recognised that the genotoxic potential of formulations should be further addressed and other endpoints should be clarified, such as long-term toxicity and carcinogenicity, reproductive/developmental toxicity and endocrine disrupting potential of formulations (European Food Safety Authority 2015a).

Endocrine disrupting properties

The endocrine disruption potential of glyphosate/GBH has not been fully assessed using the updated test guidelines that include specific endocrine endpoints, but scientific experimental studies indicate ED properties. Thus, glyphosate (alone) was reported to interact with the estrogen receptor and induce estrogenic activity in breast cancer cells (Thongprakaisang et al. 2013), GBH (Roundup) inhibited steroidogenesis by disrupting StAR protein expression in testicular Leydig cells (Walsh et al. 2000a), and glyphosate and GBH reduced the conversion of androgens to oestrogens by inhibiting the enzyme aromatase with formulations causing a stronger effect (Defarge et al. 2016; Richard et al. 2005). In animal studies in rats, gestational glyphosate exposure (50 mg/kg bw/day) caused disrupted gonadotropin expression and disturbed reproductive development and altered mating behaviour in male offspring (Romano et al. 2012) and gestational GBH exposure caused decreased lower sperm production in male offspring during adulthood (Dallegrave et al. 2007).

Postnatal GBH exposure changed the progression of puberty and caused reduced testosterone production in males (Romano et al. 2010). In females, postnatal GBH exposure caused morphological changes and alterations in expression proteins involved in uterine development (Guerrero Schimpf et al. 2017), enhanced sensitivity of the uterus to estradiol by modulating the expression of estrogen-sensitive genes (Guerrero Schimpf et al. 2018; Varayoud et al. 2017), and higher post-implantation embryo loss (Ingaramo et al. 2016). Glyphosate is included as a potential ED cat. 2 on the TEDX-list.

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Neurotoxicity

The neurotoxic potential of glyphosate has also not been assessed in regulatory studies despite some evidence of neurotoxic effects from the academic literature. These studies show that glyphosate affected the axonal differentiation and growth of cultured neurons (Coullery et al. 2016) and induced behavioural changes (hypoactivity) and alterations in dopaminergic markers in adult rats (Hernandez-Plata et al. 2015). Some epidemiological studies have reported associations between maternal peri-conceptional residential proximity to GBH sprayed crops and increased odds of neural tube defects (Rull et al. 2006), paternal occupational GBH exposure and higher risk of abortion (Arbuckle et al. 2001), and higher risk of ADHD in children of male pesticide applicators who had applied GBH (Garry et al. 2002) while other studies did not find associations with adverse pregnancy outcomes (de Araujo et al. 2016).

Gut microbiota

Besides, glyphosate has known antibacterial properties and has been reported to affect the gut microbiota of farm animals, i.e., laboratory studies where pathogenic bacteria were less inhibited by glyphosate than non-pathogenic bacteria (Ackermann et al. 2015; Kruger et al. 2013; Shehata et al. 2013). Glyphosate is also known to bind essential metals such as manganese, zinc, and cobalt which may affect mineral status as suggested by a study where glyphosate in the urine of Danish cows occurred concurrent with low levels of cobalt and manganese in the blood (Krüger et al. 2013). Both these properties might have secondary effects on health.

Co-formulants

Generally, GBHs (Roundup) are mixtures of 36-48% glyphosate, water, salts, and 10-20% adjuvants such as polyethoxylated alkyl amines (POEA) (Defarge et al. 2018) but the composition vary between different brands. Glyphosate is never used without its adjuvants, which allow and enhance its herbicidal activity by promoting its uptake and toxicity. However, adjuvants are declared as inert ingredients and classified as confidential. However, there is convincing data available that the toxicity of GBH-formulants is higher than that of glyphosate alone either because the adjuvants enhance the toxicity of glyphosate or because of their own toxic properties as demonstrated for POEA (Defarge et al. 2016; Defarge et al. 2018; Mesnage et al. 2013). The variability in adjuvants between formulations hamper the possibilities to compare results between studies unless exactly the same GBH-formulations have been used.

20.1.1.4 Polyethoxylated tallow amine (POEA)

POEA belongs to a group of petroleum-based oxidised substances used as surfactants, which are present in many GBHs and there is strong evidence that POEA surfactants decisive increase the toxicity of these formulations (Defarge et al. 2018; European Food Safety Authority 2015b). Thus, POEA-containing formulations had higher toxicity for all investigated outcomes than glyphosate alone and the conclusion from a statement from EFSA in 2015 was: "Concerns were highlighted for its genotoxic potential regarding DNA damage at concentrations not causing cytotoxicity; potentially severe adverse effects were reported with regard to the reproductive and developmental toxicity which identify the need to investigate the potential for endocrine disruption of POE-tallowamine.

No data are available regarding long-term toxicity and carcinogenicity, and developmental toxicity was not investigated in a second species (rabbits)" and " The genotoxicity, long-term toxicity and carcinogenicity, reproductive/developmental toxicity and endocrine disrupting potential of POE-tallowamine should be further clarified. There is no information regarding the residues in plants and livestock. Therefore, the available data are insufficient to perform a risk assessment in the area of

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human and animal health for the co-formulant POE-tallowamine” (European Food Safety Authority 2015b). According to the Rapporteur Member State for glyphosate, Germany, POEA should be classified and labelled for acute oral toxicity Tox. 4, H302, 'Harmful if swallowed', for skin and severe eye irritation, as Skin Irrit. 2, H315, 'Causes skin irritation', and Eye Dam. 1, H318, 'Causes serious eye damage' and skin sensitisation Skin Sens. 1, H302, 'May cause an allergic skin reaction' according to CLP criteria. Most likely, classification for inhalative toxicity would be also needed (European Food Safety Authority 2015b). A recent study found higher toxicity of POEA than of glyphosate in both plant and mammalian cells and stronger inhibition of the enzyme aromatase, which converts androgens to estrogens, and is a marker of cellular ED properties (Defarge et al. 2018). POEA is only one out of many adjuvants used in pesticide formulations as solvents, surfactants, antifoaming agents etc. Many of these substances have toxic properties (Mesnage and Antoniou 2017; Székács 2017) and may add to or enhance the toxicity of the “active” ingredient.

However, they are generally not included in the risk assessment of long-term health effects or included in surveys of dietary exposure to pesticide residues or HBM studies. This data gap represents an important source of error and may result in underestimation of health risk related to pesticide exposure.

20.1.1.5 Fipronil

Fipronil (IUPAC: (±)-5-amino-1-(2,6-dichloro- α,α,α -trifluoro-para-tolyl)-4-trifluoromethylsulfinyl-pyrazole-3-carbonitrile) is a phenylpyrazole insecticide. In insects, fipronil or its major metabolite (fipronil sulfone) noncompetitively binds to GABA_A-gated chloride channels, thereby blocking the inhibitory action of GABA_A in the central nervous system (CNS). This leads to hyperexcitation at low doses, and paralysis and death at higher doses. Fipronil exhibits a >500-fold selective toxicity to insects over mammals, primarily because of affinity differences in receptor binding between insect and mammalian receptors. However, this selectivity is less pronounced for fipronil metabolites (sulfone and desulfinyl) and especially fipronil-sulfone is reported to be twenty times more active at mammalian chloride channels than at insect chloride channels (Zhao et al. 2005). It should also be emphasised that fipronil-sulfone is rapidly formed in humans and experimental animals and persist much longer in the body than fipronil. The toxicity of another metabolite, fipronil desulfinyl, is qualitatively similar to that of fipronil, but the dose-effect curve for neurotoxic effects appears to be steeper for fipronil desulfinyl. Also, fipronil desulfinyl appears to have a much greater affinity to bind to sites in the chloride ion channel of the rat brain GABA receptor. This finding appears to be consistent with the greater toxicity of fipronil desulfinyl in the CNS of mammals. Therefore, toxic effects in mammals are likely due to the sulfone metabolite and to the primary environmental metabolite (photoproduct) fipronil-desulfinyl. Fipronil elicits neurotoxicity in mammals by inhibition of GABA-gated chloride channels, producing hyperexcitability of the central nervous system (Gupta and Milatovic 2014). Accordingly, fipronil is classified as STOT-RE 1 (H372) “Causes damages to organs through prolonged or repeated exposure” for the nervous system (table 1). Fipronil has also been reported to be a developmental neurotoxicant and to induce thyroid disruption in rats (Gupta and Milatovic 2014) and fipronil has been included in the TEDX list since 2011 and in the EU impact report as potential endocrine disruptor Cat. 2 (JRC) (EC 2016).

Besides, the US EPA has classified fipronil as “Group C - Possible Human Carcinogen” based on increases in thyroid follicular cell tumours in both sexes of the rat (Jackson et al. 2009), but fipronil did not show genotoxicity/mutagenicity potential in a battery of in vitro and in vivo tests (EU Standing Committee on Biocidal Products 2011).

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20.1.1.6 Possibility of mixture effects

The general population is exposed to a mixture of many different pesticides from the diet and occupational exposure settings will also often include mixtures of pesticides. Many currently used pesticides possess neurotoxic and/or endocrine disrupting properties and although the exposure level to the individual pesticides is low, exposure to several pesticides with similar mode of action (e.g., different pyrethroids) or same target organ (e.g., the nervous system for pyrethroids and OPs) are likely to be additive. Monitoring pesticides through HBM4EU will help describe the aggregated exposure of the general EU population. Such data can contribute to the EuroMix project (<https://www.euromixproject.eu>) which aims at developing a strategy for refining future risk assessment of mixtures relevant to national food safety authorities, public health institutes, the European Food Safety Authority (EFSA), the European Chemical Agency (ECHA), industry, regulatory bodies and other stakeholders.

Many epidemiological studies provide evidence of adverse health effects related to mixtures of pesticides although individual pesticides or pesticide groups could not be pinpointed. One example is a study among women undergoing infertility treatment, and for whom intake of fruit and vegetables with high content of pesticide residues was found to be associated with lower probabilities of pregnancy and live birth (Chiu et al. 2018a) whereas in men, intake of fruit and vegetables with low pesticide content was associated with higher total sperm count and sperm concentration (Chiu et al. 2016). Assessment of pesticide exposure in these studies was based on data obtained from food frequency questionnaires combined with surveillance data on pesticide residues in commodities. This approach was previously validated by comparing the results with biomonitoring data showing that higher intake of high-pesticide residue fruit and vegetables was associated with higher urinary concentrations of metabolites of organophosphate and pyrethroid insecticides and the phenoxy acetic acid herbicide 2,4-D (Chiu et al. 2018b), all of which are frequently detected in fruit and vegetables at the European market (European Food Safety Authority 2017). The risk is especially high if exposure occurs during vulnerable time periods in foetal life or childhood. Thus, maternal occupational exposure to mixtures of pesticides, in the first trimester before the pregnancy was recognised, was found associated with impaired reproductive development in the boys (Andersen et al. 2008; Wohlfahrt-Veje et al. 2012a), earlier puberty and impaired neurobehavioral function in the girls (Andersen et al. 2015; Wohlfahrt-Veje et al. 2012b), and lower birth weight followed by increased body fat accumulation during childhood (Wohlfahrt-Veje et al. 2011).

20.1.2 Exposure characteristics

20.1.2.1 Trends in production volume and environmental/food concentrations

In 2015, the countries with the highest pesticide sale per hectare of agricultural land were Malta, the Netherlands, Cyprus, Belgium, Ireland, Italy and Portugal. These countries were above 5 kg of pesticide active ingredient/ha, with Malta at 15 kg active ingredient/ha. The EU average was 3.8 kg of pesticide active ingredient/ha. (calculated by EEA based on Eurostat data for pesticide sales (see: <https://www.eea.europa.eu/airs/2017/environment-and-health/pesticides-sales>).

Data for the sale of specific groups of pesticides in the EU is available only for 2016 (Eurostat) and therefore time trends in sale cannot be evaluated. For pyrethroids, 965 tons (active substance) were sold in 17 of the member states (no available data for 3 and confidential data from 8 countries). For organophosphate insecticides, 2736 tons (active substance) were sold in 12 of the member states (no available data for 5 and confidential data for 11 countries) and almost half (1159 tons) of the OPs were sold in Poland (Eurostat). No specific data on the sale of glyphosate or fipronil is available.

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For the general population, pesticide residues in food constitute the main source of exposure. This has been illustrated in intervention studies where the urinary excretion of pesticides reduced markedly after one week of limiting consumption to organic food (Bradman et al. 2015; C Lu et al. 2006; Liza Oates et al. 2014). Similar conclusions have emerged from studies investigating associations between urinary concentrations of pesticides and questionnaire information on food intake and organic food choices. Thus a high intake of fruit and vegetables is positively correlated with pesticide excretion (Berman et al. 2016; Ye et al. 2015) and frequent consumption of organic produce is associated with lower urinary pesticide concentration (Berman et al. 2016; Curl et al. 2015).

Children have higher food intake per kg body weight leading to higher exposure levels as also confirmed in most HBM-studies. Besides, non-dietary sources (e.g., residential use or living in the vicinity of pesticide treated crops) can also be important determinants of exposure (Babina et al. 2012; Curl et al. 2015; Curwin et al. 2007; Dereumeaux et al. 2018; Fortes et al. 2013; Glorennec et al. 2017; CS Lu et al. 2006; L. Oates et al. 2014; Roca et al. 2014; Ye et al. 2015).

The EFSA reports on pesticide residues in food samples collected in 2015 (published in 2017) and in 2016 (published 2018) shows the combined results from the coordinated control programme (EUCP) and the national control programmes (NP) from the member states, Iceland and Norway (European Food Safety Authority 2017, 2018). Several pyrethroids, chlorpyrifos, dimethoate, and glyphosate were quantified in more than 1% of the plant products analysed.

Baby food products are included in the control programs and the Commission has defined specific rules for foods specially manufactured for infants (below 12 months of age) and young children (between 1 and 3 years of age) in Directive 2006/141/EC. It requires that infant formula and follow-on formula contain no detectable levels of pesticide residues, meaning not more than 0.01 milligrams of pesticide residues per kilogram. The Directive also prohibits the use of certain very toxic pesticides (including omethoate and a few other organophosphates) in the production of infant and follow-on formulae and establishes levels lower than the general maximum level of 0.01 milligrams per kilogram for a few other very toxic pesticides (including fipronil and some organophosphates). However, the pesticide content in human breast milk is not covered by the control programs, and while persistent organochlorine pesticides are commonly detected in breast milk, only few studies have included lipophilic pesticides in current use. In studies from the US, the detection frequency for chlorpyrifos in human milk samples collected between 2007 and 2011 was 100% with median concentrations of 0.06 ng/ml milk (Chen et al. 2014) and 0.03 ng/g milk (Weldon et al. 2011). Pyrethroids were not detected in human milk samples (n=10) in one of these studies (Chen et al. 2014) while permethrin was detected in all samples from the other study in concentrations of approximately 0.10 ng/g milk (Weldon et al. 2011). The pyrethroids, cypermethrin, lambda-cyhalothrin, permethrin and deltamethrin were detected in all human milk samples collected between 2009 and 2010 in Brazil (n= 20), Columbia (n=27) and Spain (n=6) from areas without pyrethroid use for malaria control (Corcellas et al. 2015). In samples (n=127) from Punjab in India, a median concentration of cyfluthrin of 189 ng/g milk and a max concentration of 4.1 mg/g milk was reported (Sharma et al. 2014).

Since breastfeeding is known to confer numerous long-lasting benefits to infants (Victoria et al. 2016) and exclusively breastfeeding for the first 6 months therefor is recommended by WHO, it is important to limit contaminants in breast milk that might compromise the health benefits. Thus, knowledge on pyrethroid and chlorpyrifos concentrations in human breast milk collected in the EU would provide information on the risk of lactational transfer to infants for these substances.

Due to neurotoxic and endocrine disrupting properties of most of the included pesticides, pregnant women and children are considered the most vulnerable population groups. So far only studies

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based on the PELAGIE cohort in France mentioned above have addressed associations between urinary levels of pyrethroid and OP metabolites and child neurodevelopment at exposure levels occurring in the general population. The exposure levels measured for both OPs and pyrethroids in the PELAGIE cohort were considerably lower than reported from other cohorts and more representative exposure levels for EU citizens are needed to characterise the risk of adverse effects on neurodevelopment in European populations.

Population groups with higher exposure levels than the general population will also have enhanced risk of adverse health effects. Among these are agricultural workers who mix and/or apply pesticides onto crops and or handle the crops/plants after treatment, and workers employed in companies applying biocides in residents and institutions. These groups might have high dermal and inhalation exposure.

20.1.2.2 Pyrethroids

Pyrethroids compose a large class of insecticides used to control a wide range of insects both as components of plant protection products (PPP) as insecticides and of biocidal products (BP) for wood preservation (T8) and to combat insects in animal facilities, indoors in public and commercial buildings (e.g., warehouses and hotels) as well as dwellings (T18). Some pyrethroids are also used in veterinary medicinal products and applied on animals (livestock, pets) and for treatment of scabies and head lice in humans. Currently, 16 different pyrethroids are approved as either PPP (n=13) or BP (n=13) or both (n=7), but authorisation status differs between member states. Besides, some additional pyrethroids (n=5) are under review for use as BP. Thus, the potential for human exposure is high both from intake of residues in food items and by dermal and inhalation exposures via direct contact and from dust.

Residues in food

Pyrethroid are lipophilic substances and several of the pyrethroids meet some of the criteria of the REACH Annex XIII regulation for persistency and/or bioaccumulation and are potential candidates for substitution under the Pesticides Regulation (EC) No 1107/2009 (List of candidates for substitution (Draft, January 2015)). Among these are bifenthrin, esfenvalerate, etofenprox, and lambda-cyhalothrin. Also, the co-formulant PBO meets the criteria for being very persistent (vP) according to Annex XIII to REACH (Reg 2016/2288 (EU)). Accordingly, pyrethroids have been detected in both fishes and marine mammals (Alonso et al. 2012). A recent study from Spain found pyrethroids in 100% of tissue samples collected from riverine fish (Corcellas et al. 2015). Currently, MRLs for pyrethroids in fish products have not been established in the EU.

In food items of plant origin, residues of bifenthrin, cypermethrin and lambda-cyhalothrin were the most frequently detected in 2015 (European Food Safety Authority 2017) and cypermethrin, deltamethrin, etofenprox, and lambda-cyhalothrin were the most frequently detected in 2016 (European Food Safety Authority 2018). In addition, cypermethrin and permethrin were quantified in few samples of food products of animal origin covered by the EUCP (butter and eggs in 2015 and milk and swine fat in 2016). As mentioned above, pyrethroids have been detected in human breast milk from the US, India, and South America (including six samples from Spain) but the European level is unknown at present.

Dermal and inhalation exposure

Besides dietary exposure, studies from the US have demonstrated that residential use of pyrethroids can contribute markedly to the internal exposure. Hence, floor wipe concentrations for pyrethroid insecticides were found to be significant predictors of child creatinine-adjusted urinary metabolite concentrations (Trunnelle et al. 2014b).

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A review of 15 studies in the US that examined children's exposure to pyrethroids concluded that children were exposed to pyrethroids from several sources including food, dust, and/or on surfaces at residences and for children living in homes with frequent pesticide applications dermal and inhalation exposure routes might exceed the exposure from dietary ingestion (Morgan 2012).

Most pyrethroids are rather stable in the indoor environment and increased content of pyrethroids in dust has been found more than one year after application (Leng et al. 2005).

HBM data

The exposure level to pyrethroids is likely increasing because they have replaced organophosphate and carbamate insecticides in biocides and also, to some degree, as insecticides in agriculture. HBM data are available from studies in many countries including USA, Canada, China, Japan and also from a few EU countries (France, Poland, Denmark, UK, Germany, and Spain) (Dalsager et al. 2018; Dereumeaux et al. 2018; Roca et al. 2014; Schulz et al. 2009; Viel et al. 2015; Wielgomas et al. 2013) but EU-wide data are not available. The studies from EU indicate widespread exposure to pyrethroids within the general population, including pregnant women and children but also some difference in exposure levels between countries and population groups (see Table 1 in Annex 1). Including urinary concentrations of the common pyrethroid metabolite 3-PBA in HBM4EU will provide an estimate of the aggregated exposure to pyrethroids and allow comparison with other studies and with levels associated with adverse health outcomes. That is important from a risk assessment point of view, since so many different pyrethroids are used, they replace each other, and their effects are likely additive. Besides, it will be valuable to include specific metabolites of the most used pyrethroids to get information on exposure levels while for pyrethroids that are used only to a lesser extent, detection frequency will be low, and measurements will not provide useful information.

Pyrethroids are lipophilic substances, and their concentration in human breast milk samples was inversely associated with the number of pregnancies (Corcellas et al. 2015; Sharma et al. 2014). This might indicate some accumulation in fat tissue in humans at continuous exposures as also predicted from toxicokinetic modelling (Cote et al. 2014) and mentioned in the EFSA risk assessment for e.g., bifenthrin: "Potential for accumulation in fat, terminal half-life of up to 51 days" and "Elimination complete within 48 hours, urine (13-25%) and faeces (63-88%), 3% remained in tissues and organs". Excretion via breast milk would be a potential risk for breast-feeding infants and therefore analysis of human breast milk samples would be relevant.

Thus, urinary concentrations of pyrethroid metabolites will reflect the current body burden which might depend on number of pregnancies/breast feeding periods and BMI/body fat content but such associations have not yet been explored in humans, except for one recent study reporting higher urinary 3-PBA concentrations among primiparous women compared to women with previous pregnancies and a positive association between 3-PBA and pre-pregnancy BMI (Dalsager et al. 2018).

No HBM studies including PBO were identified. After dermal application to the arms of human volunteers, about 2% of the dose was absorbed (Selim et al. 1999). The percutaneous absorption when applied to the scalp was found to be 8.3% (DrugBank). The fraction absorbed after oral exposure is less clear but was reported to be low with 64-80% excreted in faeces. After absorption, PBO is partly metabolised (the fraction is unclear) and excreted unchanged and as different metabolites in urine. PBO was not detected in any urine samples analysed at Environmental Medicine at SDU (DK) although one or more pyrethroid metabolites were detectable in all samples (unpublished results). PBO was detected in one child urine sample (3.8 µg/L) out of 14 from an agricultural population in Spain (Cazorla-Reyes et al. 2011).

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20.1.2.3 Organophosphates – chlorpyrifos and dimethoate

Chlorpyrifos, chlorpyrifos-methyl, and dimethoate are authorised as insecticide and acaricide according to Reg. (EC) No. 1107/2009 in 20, 17 and 23 of the member states, respectively. Chlorpyrifos is one of the most commonly used pesticides in the EU and worldwide. None OPs are approved as biocides in the EU.

Residues in food

Chlorpyrifos, and also dimethoate, are commonly detected in commodities produced in Europe as well as in commodities imported from third countries. Exceeding of MRLs are frequently reported for both substances, and also exceeding of acute reference values (ARfd) has been reported for chlorpyrifos based on exposure levels calculated from dietary intake estimates (European Food Safety Authority 2017). In 2014 the ADI for chlorpyrifos was reduced by a factor 10 to 0.001 mg/kg bw/day and accordingly MRLs for chlorpyrifos were lowered for many crops during 2016 leading to a higher number of exceedances in 2016 (59 exceedances out of the 10,212 samples analysed for this pesticide). In addition, a number of MRL exceedances were reported by France for dimethoate in tomatoes produced in the Mayotte oversea territory (32 exceedances in 9,618 samples reported) (European Food Safety Authority 2018). MRLs for chlorpyrifos for more commodities have been lowered during 2018. For dimethoate, the long-term dietary exposure assessment was calculated to be 101 and 6.1 % of the ADI for upper-bound and lower bound scenarios, respectively. The corresponding values for chlorpyrifos was 45.8 and 12.6 % (European Food Safety Authority 2018)

HBM data

There are some EU HBM studies including OPs but few of these were performed after 2010. (Annex 1, Table 2). Since restrictions have been imposed on the use of OPs both at EU and national level the exposure levels might be lower today, especially in countries with most restrictions on their usage. Most studies have used unspecific urinary organophosphate metabolites, i.e., dialkyl phosphates (DAPs) as a marker for the total OP exposure level. DAPs are divided into group-specific metabolites: diethyl phosphates (DEPs) and dimethyl phosphates (DMPs). DEPs include chlorpyrifos while DMPs include chlorpyrifos-methyl and dimethoate. The studies indicate wide variation in exposure level across countries and population groups. Relatively few EU HBM studies have included the metabolite 3,5,6-Trichlor-2-pyridinol (TCPY), which is specific for chlorpyrifos and chlorpyrifos-methyl (Annex 1 Table 3). No EU studies have included urine concentrations of dimethoate or its specific metabolite omethoate. Omethoate is rapidly metabolised to unspecific DMPs and only a minor fraction (approx. 1 %) is excreted in urine as dimethoate and omethoate. Accordingly, very low detection frequencies (< 1%) for dimethoate and omethoate was reported in NHANES from the US. Including DAPs in HBM4EU will allow comparison with previous studies and analyses of time-trends. Besides, it will provide an estimate of the total exposure to OPs which is likely more relevant for potential health risks than the exposure level to individual OPs, since OPs are assumed to act additively because of similar mode of actions.

20.1.2.4 Glyphosate and POEA

Glyphosate is the ISO common name for N-(phosphonomethyl)glycine (IUPAC) and a range of different salt derivatives of glyphosate are used in GBH-formulations.

Since the late 1970s, the volume of GBHs applied world-wide has increased approximately 100-fold, especially after the introduction of genetically modified plants tolerant to glyphosate, and GBHs are the most used pesticide formulations in the EU and worldwide. The estimated global use of glyphosate (as active ingredient (a.i.)) was 825.804 tons in 2014 (Benbrook 2016). The current

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sale of glyphosate in the EU cannot be extracted from Eurostat but it likely contributes the major part of “other herbicides” of approx. 65.210 tons (a.i.) in 2016 (Eurostat).

Residues in food

Application of GBHs on crops result in residues of glyphosate and its primary degradation product, aminomethyl phosphonic acid (AMPA) in food items and especially the use for pre-harvest treatment (desiccation) has probably led to higher content in food items. In the EU survey of pesticide residues in food for 2016, 3.6% of the samples analysed for glyphosate contained quantified residues. The highest quantification rate was observed for dry lentils (38%), linseeds (20%), soya beans (16%), dry peas (12%) and tea (10%). In cereals, glyphosate was mainly found in buckwheat and other pseudo-cereals (24%), followed by barley (19%), millet (18%), wheat (13%) and rye (4%). Among the 6,761 samples analysed, 19 samples (0.28%) exceeded the MRL for glyphosate (European Food Safety Authority 2018). Although AMPA has been assessed to present a similar toxicological profile to glyphosate and to apply to the same health guidance value (e.g. ADI) as glyphosate, neither AMPA nor N-acetyl-glyphosate (NAG) and N-acetyl-AMPA are included in the food surveys. NAG and N-acetyl-AMPA were identified as relevant compounds in plant/livestock residues where glyphosate tolerant genetically modified (GM) plant varieties are eaten by humans or farm animals. Accordingly, EFSA has proposed a residue definition for glyphosate for risk assessment as: - ‘sum glyphosate, N-acetyl glyphosate, AMPA and N-acetyl-AMPA expressed as glyphosate’ (European Food Safety Authority 2018). Besides, adjuvants in pesticide formulations are not included in the food surveys and therefore no data on POEA in food items are available.

Other exposure sources

Besides exposure from residues in food, the population can be exposed to GBHs from contamination of water supply (mainly AMPA), use for home gardening and from drifting from agricultural areas for residents close to treated fields. Additionally, field workers (sprayers and re-entry workers) and bystanders (including farm families) are expected to be exposed as well via the dermal route and via inhalation.

Because of concerns about the health and environment effects of glyphosate, numerous measures have been taken at the national and municipality level in order to restrict the use of GBHs. Besides, POEA has been banned from glyphosate-containing products since 2016 (https://ec.europa.eu/food/plant/pesticides/glyphosate_en) and will be put on the ‘negative list’ (chemicals not to be used in formulations of plant protection products) that is being set up in the EU. These measures are expected to affect the population's exposure to glyphosate and POEA, but since POEA has been reported to be rather persistent in agricultural soils (Tush and Meyer 2016) and is still approved in countries outside the EU, the substance may still be present in food items although there is no available information regarding residues in plants and livestock.

After the ban of POEA in GBHs at the EU market, it will likely be substituted by replacement surfactants. Thus, it might be important to monitor both POEA and future substitute substances in both food items and human samples.

HBM4EU research may be focused on the development of suspect screening approaches of POEA and eventually other relevant surfactants, permitting to generate a first level of exposure data enabling documentation of human exposure to better justify further investment in a full quantitative and validated method development.

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HBM data

Glyphosate is rapidly but incompletely absorbed after oral administration (around 20 % of the administered dose based on urinary excretion after 48 hours and comparison of kinetic behaviour after oral and iv administrations), being mostly eliminated unchanged via faeces. Absorbed glyphosate is poorly metabolised, widely distributed in the body, does not undergo enterohepatic circulation and is rapidly excreted unchanged in urine; showing no potential for bioaccumulation (European Food Safety Authority 2015a). Humans are also exposed to AMPA and both glyphosate and AMPA have been measured in human urine samples and seem to be ubiquitous in human urine. However, only limited HBM data are available from the US (Curwin et al. 2007; Mills et al. 2017; Niemann et al. 2015) and Europe (Connolly et al. 2018; Conrad et al. 2017; Knudsen et al. 2017) (see Annex 1 Table 4) although GBHs has been widely used for many years. US levels seem higher than those seen in Europe. In a study from California, the urinary concentrations of both glyphosate and AMPA among adults had increased considerably between 1993 and 2016 (Mills et al. 2017). For further elucidation of the variation in the population's exposure and time trends, the German Environment Agency is analysing morning urine samples acquired in the cross-sectionally designed population-representative German Environmental Survey for Children and Adolescents (GerES 2014–2017) for glyphosate and AMPA. A recent study from Ireland, reported higher urinary glyphosate concentrations among horticulturalists using GBHs with peak levels up to 3 h after completing the application (Connolly et al. 2018). No HBM data for POEA are available.

So far, no epidemiological studies on GBH-related health effects using HBM exposure data have been published. The scientific community has raised concerns on the safety of glyphosate and glyphosate-based products, and there is a need for HBM data for glyphosate and its metabolites to characterise the exposure situation in the population, HBM-based epidemiological studies on potential related health effects, especially among occupationally exposed agricultural workers, pregnant women and their children and more evaluations of GBH-formulations, recognising that these mixtures likely have effects that are not predicted by studying glyphosate alone.

20.1.2.5 Fipronil

Fipronil is approved in the EU as an active biocidal agent (BP T18) used for ant and cockroach control. Only professional use indoors by application in locations normally inaccessible after application to man and domestic animals has been addressed in the EU risk assessment (Dir 2011/79/EU). It is also authorised in the EU as veterinary medicine in two products (EMA) and in more products at Member State level. As example, fipronil is the active ingredient of one of the popular ecto-parasiticide veterinary products, Frontline, which is commonly used on pets to kill fleas, and all stages of ticks. Until 2017 fipronil was also approved as insecticidal pesticide in plant protection products. A recent (2017) case of illegal use of non-approved veterinary medicinal products in poultry farms caused a large-scale contamination of eggs in several EU-countries and fipronil was detected in quantities between 0.0031 and 1.2 mg/kg (ppm) in eggs in several EU countries.

Fipronil is rapidly and extensively absorbed after oral intake and an uptake of approximately 90% has been estimated. Uptake after dermal exposure was 0.1-10% dependent on the concentration and duration of exposure (Jackson et al. 2009). After absorption, fipronil is metabolised to fipronil-sulfone and fipronil and especially the sulfone metabolite persists in in the body (especially in fatty tissues, but also in brain, liver, kidney, and adrenals) for weeks. Thus, the half-life of fipronil-sulfone in blood is long (6-10 days) reflecting a slow release of the metabolite from fat tissue (Gupta and Milatovic 2014; Jackson et al. 2009).

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Since fipronil is used as insecticide in agriculture outside the EU, residues may occur in imported commodities. In the 2016 EU survey of residues in food, fipronil (sum of fipronil and sulfone metabolite) was quantified in 57 out of 51430 analysed samples (0.11 %), 44 samples exceed the MRL and 40 of these samples were from third countries outside the EU (European Food Safety Authority 2018). Besides, pet owners and especially their children, professional biocide applicators, residents in buildings after treatment, and veterinary personal using fipronil-containing products can be exposed. However, no HBM data are available from the EU to evaluate exposure level and how widespread the exposure is after authorised uses. In a US study fipronil sulfone was present in the serum of approximately 25% of the samples (at concentrations ranged from 0.1 to 4 ng/mL) collected from volunteers (n=96) with no known pesticide/biocide exposure. In contrast no fipronil metabolites were detected in the urine samples (McMahen et al. 2015).

To investigate the transfer of fipronil from dogs treated with a spot-on product (Frontline containing 9.8% fipronil), Frontline (1.34 ml) was applied topically on adult household dogs and gloves worn for 5 min during petting were collected 24 hr and 1-, 2-, 3-, 4- and 5-weeks post-Frontline application for fipronil residue determinations using GC/MS. The highest concentration of fipronil (589 ± 206) was detected 24 h after Frontline application and decreased steadily over time to 448 ± 118 ppm after 8 days, and were undetectable after 36 days (Jennings et al. 2002). A recent study estimated the acute post-application absorbed doses to be as high as 0.56 $\mu\text{g}/\text{kg}/\text{day}$ for toddlers in households with treated pets based on current US EPA standard operating procedures (SOPs) (Cochran et al. 2015). Only one study investigating fipronil exposure among pet owners have included HBM the authors could not exclude contamination of some urine samples and therefor the HBM results were not presented (Dyk et al. 2012). Thus, especially small children with close contact to treated pets might be relatively high exposed. Further, repeated exposure among veterinary personnel who handle many dogs/cats daily, require proper protection to avoid cumulative exposure. More HBM studies are needed to characterise the exposure level for these groups and for the general population.

The following AELs has been proposed by French Rapporteur Member State for placing fipronil as a biocidal product on the market:

- ▶ AEL acute-term (secondary exposure) = 0.025 mg/kg bw.
- ▶ AEL medium-term (operator exposure) = 0.0035 mg/kg bw/d.
- ▶ AEL long-term = 0.0002 mg/kg bw/d.

20.1.2.6 Health based guidance values available for HBM data

No health-based guidance values (HBM-I or HBM-II) have been established for the pesticides but some national reference values RV95 have been established.

The German Human Biomonitoring Commission has established reference values (RV95) for organophosphate and pyrethroid metabolites in urine of both children 3-14 years of age and adults from the German population (Schulz et al. 2011) and the Institute of Environment and Health (IEH) from the Cranfield University has established RV95 for pyrethroid metabolites in urine of the general adult (>18 years) UK population (Bevan et al. 2013). The ongoing National Biomonitoring Programme (NHANES) in the US, are routinely measuring pyrethroid and organophosphate biomarkers (US Centres for Disease Control and Prevention 2017). For comparison RV95 values from the NHANES study are included (children 6-11 years and adults 20-59 years). No RV95 data on glyphosate and fipronil are available from the German Human Biomonitoring Commission and these substances are not included in NHANES.

Metabolite	Reference values (RV95) Urine concentration (µg/L)				
	Germany children	Germany adults	UK adults	NHANES children	NHANES adults
Sampling year	2003-06	1998	?	2007-08	2007-08
3-PBA	2	2	6.1	9.9	6.7
Trans-DCCA	2	2	1.6	4.0	5.4
Cis-DCCA	1	1	0.8	-	-
Cis-DBCA	-	-	1.6	<LOD	<LOD
DMP	75	135	-	43.3	30.3
DMTP	100	160	-	52.5	30.6
DMDTP	10	-	-	6.7	4.3
DEP	30	16	-	20.2	14.0
DETP	10	-	-	6.4	4.2
TCPY	-	-	-	6.0	5.9

Biomonitoring guidance values (BGVs) derived for chlorpyrifos based on biomonitoring data and PBPK/PD modelling of AChE inhibition was recently suggested (Arnold et al. 2015) to be 2100 µg/L and 520 µg/L urine for TCPy in adults and infants, respectively. These limits were based on 10% AChE inhibition in red blood cells (RBC) claimed to be precursor for adverse neurological symptoms and therefor used as point of departure. However, epidemiological studies have raised concern that this limit is not protective for neurodevelopmental effects e.g., by the US EPA (Britton 2016; Drew et al. 2016). Recently a new approach for Benchmark Dose estimation using PBPK/PD modelling and a novel pharmacodynamic (PD) dose–response model was suggested.

Simulated peak brain chlorpyrifos concentrations, were used to develop a dose–response model to predict chlorpyrifos-induced spatial memory deficits and a 15% cognitive deficit was used as point of point of departure leading to lower benchmark dose (reference dose) than when 10% AChE inhibition was used (Zurlinden and Reinfeld 2018). Corresponding urinary TCPy concentrations were not calculated.

Recently, a Human Biomonitoring Equivalent (BE) value for interpretation of urinary levels for 3-PBA was proposed (Aylward et al. 2018). Using the lowest (most stringent) BE value (Tier 1) or a weighted average based on information regarding relative exposure potential (Tier 2) combined with information on molar urinary excretion fraction of the metabolites led to 3-PBA BE values of 1.7 µg/L (Tier 1) and 87 µg/L (Tier 2).

Urinary pyrethroid and organophosphate (alkyl diphosphate) metabolites are included in the German External Quality Assessment Scheme (G-EQUAS).

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20.1.3 Policy relevance

Plant protection products (all substances except permethrin (and other pyrethroids not approved for PPP as indicated in Table 1) and fipronil) are regulated under Regulation (EC) 1107/2009. Under this regulation, the pyrethroids bifenthrin, esfenvalerate, etofenprox, lambda-cyhalothrin as well as dimethoate and fipronil are included in the draft list of candidates for substitution (January 2015).

Fipronil and pyrethroids approved as biocides are regulated under Regulation (EC) 528/2012.

Permethrin and fipronil are also used in medicinal products for human and veterinary use and regulated under Regulation (EC) 726/2004.

Residues of all the substances in food and feed is regulated under Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC.

Specific rules on the presence of pesticides residues in infant and follow-on formulae are regulated by Directive 2006/141/EC (Annex VIII) which also encompasses the rules, previously set out in Commission Directive 1999/50/EC. It requires that infant formula and follow-on formula contain no detectable levels of pesticide residues, meaning not more than 0.01 milligrams of pesticide residues per kilogram. The Directive also prohibits the use of certain very toxic pesticides in the production of infant and follow-on formulae and establishes levels lower than the general maximum level of 0.01 milligrams per kilogram for a few other very toxic pesticides. Omethoate, an OPs but also a metabolite of dimethoate, is one of the pesticides prohibited for use in the production. For fipronil the MRL is set to 0.004 mg/kg food produced for infants/young children.

Classifications of the substances related to human health outcomes according to Regulation EC 1272/2008 are shown in Table 1.

20.1.4 Technical aspects

- ▶ Biomarkers available for parent compounds or metabolites in human matrices and main characteristics of analytical methods (quantitative, semi-quantitative...).

Since the pesticides included in HBM4EU are generally metabolised and excreted within few days, urine is a better matrix than blood/serum for biomonitoring studies (Barr et al. 2005; Needham and Sexton 2000; Yusa et al. 2015). Methods for measuring multiple pesticides at the same time in hair samples (including chlorpyrifos and other organophosphates and pyrethroids) have been published (Hardy et al. 2015; Lehmann et al. 2018).

An advantage is that hair samples will reflect exposure during a longer time period than urine samples, but further development and validation of the methods is needed. However, hair samples are not available in the HBM4EU alignment studies and therefore this matrix is not considered relevant. Chlorpyrifos and pyrethroids (parent compounds) can also be analysed in human breast milk samples (Chen et al. 2014; Corcellas et al. 2012; Weldon et al. 2011). This matrix is considered relevant for a pilot study if bio-banked breast milk samples are available from EU studies.

There are established, validated sensitive methods for analysing metabolites of pyrethroids, chlorpyrifos/chlorpyrifos-methyl, and organophosphates (group-specific) and glyphosate in urine samples as described below and shown in Annex 2. Harmonisation of the methods within partner countries might be necessary.

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The following urinary metabolites of pyrethroids have been used as biomarkers in most previous studies:

- ▶ 3-phenoxybenzoic acid (3-PBA) is a common metabolite of most pyrethroids and estimate for the total exposure:
- ▶ *cis*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid (*Cis*-DCCA) and *trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid (*trans*-DCCA) are metabolites of the respective isomers of permethrin, cypermethrin and cyfluthrin;
- ▶ *cis*-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid (*Cis*-DBCA) is a specific metabolite of deltamethrin,
- ▶ 4-fluoro-3-phenoxybenzoic acid (F-PBA) is a metabolite of cyfluthrin.

The urinary pyrethroid metabolites (+ the specific metabolite (TCPy) of chlorpyrifos/chlorpyrifos, see below) can be measured in a single run using high performance liquid chromatography and tandem mass spectrometry (LC-MS/MS) (Dalsager et al. 2018; Davis et al. 2013). However, LODs for the specific metabolites are lower if analysed by gas chromatograph/mass spectrometry (GC-MS) (Viel et al. 2015). The detection frequency (percentage of population with concentrations above LOD) for the specific metabolites is lower than for 3-PBA in most studies, and for some metabolites none, or only a few percent, of the samples are above LOD. Pyrethroid formulations generally consist of multiple stereoisomers with different toxicokinetic properties. Most studies report a higher concentration of *trans*-DCCA than *cis*-DCCA in urine reflecting the major exposure route. The urinary excretion pattern is affected by the exposure route with higher urinary *trans*-DCCA concentrations relative to *cis*-DCCA after oral exposure, while dermal (and probably also inhalation) exposure results in a more equal ratio (Cote et al. 2014). Besides, methods (GS-MS) for analysing specific urinary metabolites for bifenthrin, esfenvalerate, and lambda-cyhalothrin (Tao et al. 2013) and for bifenthrin combined with cyhalothrin (Bevan et al. 2013) have been described.

Day-to-day variability in individual urinary concentrations of 3-PBA has been reported to be low and much more stable than for organophosphate metabolites (Wielgomas 2013), probably because excretion from storage in fat tissue prolong the excretion time after continuous exposures (Cote et al. 2014). Inter-individual variability in urinary concentrations of the metabolites of specific pyrethroids is unknown but is likely larger.

Unspecific OP metabolites, dialkyl phosphates (DAPs), as a marker for the total OP exposure level, can be quantified in human urine using capillary gas chromatography/tandem-mass spectrometry (GC/MS/MS) (Bravo et al. 2004) (Barr, D. et al., 2010). DAPs are divided into group-specific diethyl phosphates (DEPs) and dimethyl phosphates (DMPs). DEPs include chlorpyrifos while DMPs include chlorpyrifos-methyl and dimethoate.

The specific main metabolite, 3,5,6-trichlor-2-pyridinol (TCPy) of chlorpyrifos and chlorpyrifos-methyl can be quantified in human urine using capillary gas chromatography/mass spectrometric detection (GC-EI/MS) (Koch et al. 2001) or LC-MS/MS (Dalsager et al. 2018; Davis et al. 2013).

No sensitive specific urinary biomarker is available for dimethoate, but dimethoate is metabolised to DMPs and thus included in that biomarker.

Glyphosate and the environmental metabolite AMPA can be analysed in urine by GC-MS-MS analysis (Conrad et al. 2017). Glyphosate has also been analysed in urine by ELISA and seem to be comparable with results obtained by GC-MS (high correlation) but the maximum concentrations found in human urine by the two methods differed (Krüger et al. 2014) and more validation of the ELISA method would be needed before applying this approach in a large HBM study. Glyphosate has also been determined in serum using HPLC with fluorescence detection (Kongtip et al. 2017)

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but urine is the preferred matrix for non-persistent compounds at low exposure levels as explained above.

No HBM methods for POEA, or related surfactants, are described in the literature.

Fipronil sulfone in serum seem to be the best exposure biomarker for fipronil exposure because the metabolite is rather stable and probably also the main responsible for toxic effects. A time-of-flight mass spectrometry (LC/TOF-MS) method to measure fipronil sulfone in serum and milk samples is available (McMahen et al. 2015) and an ELISA developed for the detection of total fipronil (parent compound and metabolites) in serum has also been used (Mohamed et al. 2004). Recently, a LC-MS/MS method to measure hydroxyl-fipronil in urine was developed using rat urine (Vasylieva et al. 2017) but this method has not yet been applied on human samples.

20.1.5 Societal concern

The general population is exposed to pesticide residues in the food and according to a Eurobarometer survey from 2014, 43% were worried about the impact on their health of chemicals used in everyday products and 29% were worried about agricultural pollution (use of pesticides, fertilisers etc). In a Danish survey from Beredskabsstyrelsen (2016), 30% were concerned about toxic pollutants in food and drinking water. There is a lot of media attention both in the EU and globally, in particular related to concerns about impact on health, especially related to endocrine and developmental neurotoxicity of pesticides as well as the carcinogenic potential of especially glyphosate. Glyphosate assessment by EFSA and by ECHA has generated a wide media coverage, with a wide alliance of European NGO campaigning against its reauthorisation and many municipal and regional governments taking measures to reduce its use. Also, the European Citizens' Initiative calling on the European Commission to propose to member states a ban on glyphosate, to reform the pesticide approval procedure, and to set EU-wide mandatory reduction targets for pesticide use, has collected over 1,320,517 signatures.

Also, the recent discovery of fipronil in eggs on the EU market, as a result of misuse of the active substance in chicken stable areas that were directly accessible to the chickens, gained wide attention and societal concern.

Although the regulatory risk assessment of pesticides currently practiced in the EU is comprehensive there are some concerns in the scientific community, that this risk assessment is inadequate at addressing mixed exposures, specifically for carcinogenic effects (Goodson et al. 2015), endocrine-disrupting effects (Jacobsen et al. 2012; Kortenkamp 2014), and developmental neurotoxicity (Bjorling-Poulsen et al. 2008). Furthermore, there are concerns that test protocols lag behind independent science (Beronius et al. 2013) and that studies from independent science, including epidemiological studies, are not fully considered (Tweedale et al. 2014). In 2015 a Steering Committee of scientists adapted the Intergovernmental Panel on Climate Change weight-of-evidence characterisation for probability of causation based upon levels of available epidemiological and toxicological evidence for one or more chemicals contributing to disease by an endocrine disruptor mechanism. A mean cost of €157 billion annually in EU was estimated by Monte Carlo simulations (Trasande et al. 2015). Effects on brain development are likely to be lasting and one main outcome is cognitive deficits, often expressed in terms of losses of IQ points. When US data on adverse effects on children's IQ levels was utilised to calculate the approximate costs of organophosphate exposure in the EU, the total number of IQ points lost due to these pesticides was estimated to be 13 million per year, representing a value of about € 125 billion (Bellanger et al. 2015). Although this estimate is somewhat uncertain (most likely underestimated as it focused only on one group of pesticides and on one outcome), this calculation emphasises the need to generate better and stricter safety information on pesticides, limit human pesticide exposure further through regulation and public information, obtain better exposure assessments for

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population-wide pesticide exposures, and acquire better documentation on the adverse health effects associated with current pesticide exposure.

Finally, the controversy related to glyphosate versus GBHs has emphasised the need to include the whole pesticide formulations including adjuvants in the risk assessment procedure.

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20.2 Categorisation of Substances

Table 20-1: Substances included in the substance group, listed according to availability of toxicology and human biomarker data, in category A, B, C, D, E substances (see general introduction)

Cat.	Abbrev. / Acronym	Systematic name	CAS No.	Classification (EC1272/2008) and thresholds	Regulation
B	PYR	Pyrethroids (group)*			
B		Permethrin (proposed lead substance)	52645-53-1	Acute Tox. 4 - H302; Acute Tox. 4 - H332; STOT SE 3 - H335; Skin Sens. 1 B H317; AELlong-term: 0.05 mg/kg bw/d ADI: 0.05 mg/kg bw/d (WHO/FAO JMPR) Potential ED cat.2 (JRC) (EC 2016)	Not approved as plant protection product (PPP) in EU Approved as biocidal product (BP) T8 and T18 Reg. (EU) No 1090/2014
B		Acrinathrin	101007-06-1	No classification ADI: 0.01 mg/kg bw/d; ARfD: 0.01 mg/kg, AOEL: 0.007 mg/kg bw/dg (Reg (EU) 2017/358); Identified as Potential ED cat.2 (JRC) (EC 2016)	Approved as PPP Reg. (EU) no 2017/358, No 540/2011, No 974/2011 (2008/934) Not approved as BP
B		Allethrin	584-79-2		Not approved as PPP or BP in EU
B		Alpha-cypermethrin (alphamethrin)	67375-30-8	Acute Tox 3 – H301, STOT SE 3 – H335, STOT RE 2 – H373 (nervous system) ADI: 0.015 mg/kg bw/d, ARfD: =.04 mg/kg bw, AOEL: 0.01 mg/kg bw/d (Dir 4/58)	Approved as PPP 04/58/EC, Reg. (EU) 018/917, Reg. (EU) No 540/201 Approved as BP T18 Reg. (EU) 2015/405
B		Bifenthrin	82657-04-3	Acute Tox 2 – H300, Acute tox 3 – H331, STOT RE 1 – H372 (nervous system), Skin Sens 1B – H317, Carc 2 – H351, ADI: 0.015 mg/kg bw/dg, ARfd: 0.03 m/kg b, AOEL: 0.0075 mg/kg bw/d (Reg (EU) 2018/291) Potential ED cat.2 (JRC) (EC 2016)	Approved as PPP Reg. (EU) 2017/195, Reg. (EU) 2018/291, Reg. (EU) No 582/2012 Approved as BP T8: directive 2011/10/EU

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Cat.	Abbrev. / Acronym	Systematic name	CAS No.	Classification (EC1272/2008) and thresholds	Regulation
B		Cyfluthrin	68359-37-5	Acute tox 2 – H300 ADI: 0.003 mg/kg bw/d, ARfD: 0.02 mg/kg bw, AOEL: 0.02 mg/kg bw/d (Dir 03/31) Potential ED cat.3 (JRC) (EC 2016)	Approved as PPP (as beta-cyfluthrin) 03/31/ECReg. (EU) 2017/1511Reg. (EU) No 540/2011Reg. (EU) No 823/2012 (Reg. (EU) 2016/950) Approved as BP T18 reg (EU) 2016/1937
B		Cypermethrin	52315-07-8	Acute tox 4 – H302, Acute tox 4 – H332, STOT SE 3 – H335 ADI: 0.05 mg/kg bw/d, ARfD: 0.2 mg/kg bw, AOEL: 0.06 mg/kg bw/d (Dir 05/53), ADI: 0.02 mg/kg bw/d, ARfD: 0.04 mg/kg bw (JMPPR 2006) Potential ED cat.1 (JRC) (EC 2016)	Approved as PPP 05/53/ECReg. (EU) 2017/1511Reg. (EU) No 540/2011 (Reg. (EU) No 533/2013) Approved as BP T8 Reg(EU) 945/2013 and T18 Reg (EU) 2018/1130
B		Zeta-cypermethrin	52315-07-8 (same cypermethrin) as	No classification ADI: 0.04 mg (kg bw/d, ARfD: 0.125 mg/kg bw, AOEL: 0.02 mg/kg bw/d (EFSA 08)	Approved as PPP, 2009/37 Reg. (EU) No 540/2011
B		Zyphenothrin (or cyphenothrin)	39515-40-7		Approved as BP PT18
B		d-Allethrin	231937-89-6; 584-79-2	Acute tox 4	Under review as BP T18
B		Deltamethrin	52918-63-5	Acute tox 3 – H301, Acute tox 3 – H331 ADI: 0.01 mg/kg bw/d, ARfD: 0.01 mg/kg bw, AOEL: 0.0075 mg/kg bw/d (Dir 03/5) Potential ED cat.2 (JRC) (EC 2016)	Approved as PPP 03/5/ECReg. (EU) 2017/1511Reg. (EU) No 540/2011Reg. (EU) No 823/2012. Full dossier is currently under review for renewal (AIR-3 programme). Approved as BP T18, directive 2011/81/EU
B		d-Tetramethrin	1166-46-7	Acute tox 4, Carc 2, STOT SE 2 (nervous system, inhalation)	Under review as BP T18
B		Empenthrin	54406-48-3		Under review as BP T18

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Cat.	Abbrev. / Acronym	Systematic name	CAS No.	Classification (EC1272/2008) and thresholds	Regulation
B		Epsilon-momfluorothrin	1065124-65-3	Acute tox 4, STOT SE 2 (nervous system)	Approved as BP T18 Reg (EU) 2016/2289
B		Esbiothrin	260359-57-7		Under review as BP T18
B		Esfenvalerate	66230-04-4	Acute tox 3 – H301, Acute tox 3 – H331, Skin Sens1 – H317 ADI: 0.0175 mg/kg bw/d, ARfD: 0.0175 mg/kg bw, AOEL: 0.011 mg/kg bw/d (Reg (EU) 2015/2047) Potential ED cat.2 (JRC) (EC 2016)	Approved as PPP 00/67/ECReg. (EU) 2015/2047Reg. (EU) No 540/2011 (2010/77/EU, Reg. (EU) 2015/1885)
B		Etofenprox	80844-07-1	Lact.- H362 ADI: 0.03 mg/kg bw/d, ARfD: 1 mg/kg bw, AOEL: 0.06 mg/kg bw/d (EFSA 08) Potential ED cat.3 (JRC) (EC 2016)	Approved as PPP 2009/77/ECReg. (EU) No 540/2011 Approved as BP T8 (Dir 2008/16/EC) and T18 (Reg (EU) 1036/2013)
B		Fenpropathrin	39515-41-8	Acute tox – H301, Acute tox 4 – H312, Acute tox 2 – H330, ADI: 0.03 mg/kg bw/d, ARfD 0.03 mg/kg bw (JMPPR 2012)	Not approved as PPP (Reg (EC) No 1107/2009)
B		Fenvalerate	51630-58-1	No classification ADI: 0.0125 mg/kg bw/d (EMEA)	Not Approved as PPP (98/270/EC)
B		Imiprothrin	72963-72-5	Acute tox 4	Approved as BP T18 Reg (EU) 2017/2326
B		Lambda-cyhalothrin	91465-08-6	Acute tox 3 – H301, Acute tox 4 – H312, Acute tox 2 – H330 ADI: 0.0025 mg/kg bw/d, ARfD: 0.005 mg/kg bw, AOEL: 0.00063 mg/kg bw/d (Reg (EU) 2016/146) Potential ED cat.2 (JRC) (EC 2016)	Approved as PPP 00/80/EC Reg. (EU), 016/146 Reg. (EU) No 540/2011 Approved as BP T18 (Dir 2011/10/EU)

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Cat.	Abbrev. / Acronym	Systematic name	CAS No.	Classification (EC1272/2008) and thresholds	Regulation
B		Gamma-cyhalothrin	76703-62-3	No classification ADI: 0.0012 mg/kg bw/d, ARfD: 0.0025 mg/kg bw, AOEL: mg/kg bw/d (Reg (EU) No 1334/2014.	Approved as PPP Reg. (EU) No 1334/2014
B		Metofluthrin	240494-71-7	Acute tox 3 and 4, STOT SE 1 (nervous system), STOT RE 2	Approved as BP T18 (Dir. 2010/71/EU)
B		Prallethrin	23031-36-9	Acute tox 3 and 4	Under review as PB T18.
B		Tau-fluvalinate	102851-06-9	Acute tox 4 – H302, Skin Irrit 2 – H315. ADI: 0.005 mg/kg bw/d, ARfD: 0.05 mg/kg bw, AOEL: 0.0044 mg/kg bw/d Potential ED cat.3 (JRC) (EC 2016)	Approved as PPP Reg (EU) 2011/19/ No 540/2011
B		Tefluthrin	79538-32-2	Acute tox 2 – H300, Acute tox 2 – H310, Acute tox 1 – H330 ADI: 0.005 mg/kg bw/d, ARfD: 0.005 mg/kg bw, AOEL: 0.0015 mg/kg bw/dg (EFSA 10) Potential ED cat.3 (JRC) (EC 2016)	Approved as PPP Reg. (EU) No 800/2011
B		Tetramethrin	7696-12-0	Acute Tox. 4, Carc. 2, STOT SE 2 (nervous system, inhalation)	Not approved as PPP (2002/2076) Under review as BP T18
B		Transfluthrin	118712-89-3	Skin Irrit 2	Approved as BP T18, Reg (EU) 407/2014
B		1R-trans-phenothrin (or D-phenothrin)	26046-85-5		Approved as BP T18, Dir. 2013/41/EU

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Cat.	Abbrev. / Acronym	Systematic name	CAS No.	Classification (EC1272/2008) and thresholds	Regulation
B	PBO	Piperonyl butoxide (co-formulant, synergist)	51-03-6	Carc.2; H351 (increased incidence of hepatocellular adenomas and carcinomas in mouse), STOT SE 3; H335 (respiratory tract irritation), EUH066. The long and medium term AELs and ADI are equal to 0.2 mg/kg bw/d and the AEL short term is 1.0 mg/kg bw/d. Classified as Group C Possible Human Carcinogen by US-EPA	Approved as BP T18 Reg. (EU) No 528/2012, Reg (EU) 2016/2288
B	OP	Organophosphates			
B		Chlorpyrifos (OP)	2921-88-2	Acute tox 3 – H301, ADI: 0.001 mg/kg bw/d, ARfD: 0.005 mg/kg bw, AOEL: 0.001 mg/kg bw/d (EFSA 2014) Potential ED cat.3 (JRC) (EC 2016)	Approved as PPP, 05/72/EC, Reg. (EU) No 540/2011, Reg. (EU) No 762/2013, Reg. (EU) No 84/2018. Full dossier is currently under review for renewal (AIR-3 programme). Chlorpyrifos, and chlorpyrifos-methyl, did not get renewal and their authorisations were withdrawn by 16 February 2020. Max. period of grace: 16 April 2020.
C		Dimethoate (OP)	60-51-5	Acute tox 4 – H302, Acute tox 4 – H312 ADI: 0.001 mg/kg bw/d, ARfD: 0.01 mg/kg bw, AOEL: 0.001 mg/kg bw/d (EFSA 2013). In an EFSA risk assessment published in 2018 no toxicological reference values were established due to genotoxicity concerns (European Food Safety Authority (EFSA) 2018). Potential ED cat.2 (JRC) (EC 2016).	Approved as PPP, 07/25/EC, Reg. (EU) 2018/917, Reg. (EU) No 540/2011. Full dossier is currently under review for renewal (AIR-3 programme).

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Cat.	Abbrev. / Acronym	Systematic name	CAS No.	Classification (EC1272/2008) and thresholds	Regulation
C		Fipronil	120068-37-3	Acute tox 3 – H301, Acute tox 3- H311, Acute tox 3 – H331, STOT RE – H372 (nervous system), ADI: 0.0002 mg/kg bw/d, ARfD: 0.009 mg/kg bw, AOEL: 0.0035 mg/kg bw/d (Dir 07/52) Potential ED cat.2 (JRC) (EC 2016) Classified as group C "possible human carcinogen" by US-EPA.	Not approved as PPP Reg. (EU) 2016/2035Reg. (EU) No 540/2011Reg. (EU) No 781/2013 Approved as BP T18 (Dir 2011/79/EU)
B		Glyphosate	1071-83-6	Eye Dam 1 – H318 ADI: 0.5 mg/kg bw/d, ARfD: 0.5 mg/kg bw, AOEL: 0.1 mg/kg bw/d (Reg (EU) 2017/2324) Potential ED cat.2 (JRC) (EC 2016) Classified as a "probable human carcinogen" group 2A by IARC.	Approved as PPP Reg. (EU) 2017/2324, Reg. (EU) No 540/2011 2016/1056, Reg. (EU) 2016/1313) Latest approval 17/12 2017 for 5 years, expiring 15/12 2022
C	POE-Tallowamine	Polyethoxylated tallow amine (co-formulant for glyphosate)	61791-26-2		No registration (but many pre-registrations) under REACH (According to information from ECHA January 2019).

PPP: plant protection product, BP: Biocidal product, T8: wood preservative; T18: Insecticides, acaricides and products to control other arthropods

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20.3 Policy-related questions

1. Which are the most suitable methods and biomarkers of exposure?
2. What are the current exposure levels of the EU population to the prioritised pesticides: pyrethroids, chlorpyrifos and dimethoate, glyphosate (in combination with polyethoxylated tallow amine (POEA)), and fipronil and do the exposure levels differ between countries?
3. What are the main dietary sources of exposure across the member states?
4. What are other sources and pathways of exposure?
5. What are exposure levels among occupationally exposed workers?
6. Are the exposure levels of health-relevance/concern for vulnerable groups (infants, children and pregnant women) or high exposure population groups (e.g., occupational exposure)?
7. How can cumulative risks of pesticide mixtures on health outcomes be assessed and integrated in regulation?
8. Is it possible to establish EU wide accepted health-based guidance values for the pesticides, preferably taking potential mixture effects and evidence from epidemiological studies into account?
9. How can HBM data from HBM4EU feed into prioritisation of the pesticides for risk assessments and regulatory decision-making?

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20.4 Research Activities to be undertaken

While completing this table please think of data and gaps concerning toxicology (and exposure [in three dimensions: **location** (differences between the countries), **time** (trends) and **age** (data available for which age group)]. If no HBM method is available or the method has to be harmonised within partner countries, please indicate this too.

Table 20-2: Listing of research activities to be carried out to answer the policy questions summed up in 1.3

Policy question	Substance	Available knowledge	Knowledge gaps and activities needed
1. Which are the most suitable methods and exposure biomarkers?	Cat B (pyrethroids, chlorpyrifos, and glyphosate)	<p>There are established and validated methods for analysing urinary metabolites as marker for the total pyrethroid exposure (3-PBA), the combined exposure to cypermethrin, permethrin and cyfluthrin (cis- and trans-DCCA), and for some specific pyrethroids (deltamethrin, cyfluthrin, bifenthrin).</p> <p>The detection frequency is low for most specific pyrethroid metabolites but depends on the limit of detection (LOD) which vary between different analytical approaches and labs. Furthermore, pyrethroids are often metabolised to several different metabolites with low fractions of each specific metabolite.</p> <p>There are available methods for analysing the metabolite, TCPy, which is specific for chlorpyrifos and chlorpyrifos-methyl and for group-specific urinary organophosphate metabolites, i.e., dialkyl phosphates (DAPs) as a marker for the total OP exposure level. DAPs are divided into diethyl phosphates (DEPs) and dimethyl phosphates (DMPs). DEPs include chlorpyrifos while DMPs include chlorpyrifos-methyl and dimethoate.</p> <p>A method exist to measure some pyrethroids (total and some specific) and chlorpyrifos simultaneously.</p> <p>Glyphosate is primarily excreted in urine as unchanged parent molecule. Humans are also exposed to AMPA which is the main metabolite found in water. Both glyphosate and AMPA can be measured in urine with established methods.</p>	<p>Activities:</p> <ol style="list-style-type: none"> 1. Evaluation and selection of best suited biomarkers of exposure (Y3-Y4) (WP9) 2. The methods for analysing urinary metabolites of pyrethroids, chlorpyrifos, organophosphates (DMPs and DEPs), and glyphosate, need to be harmonised within partner countries to obtain comparable values and LODs (WP9) 3. Development/validation of methods to include more specific pyrethroid metabolites could be considered based on expected prevalent exposure and whether major specific metabolites are formed (WP9).

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Policy question	Substance	Available knowledge	Knowledge gaps and activities needed
	Cat C (dimethoate, fipronil, and POEA)	<p>Because dimethoate and the specific metabolite are rapidly metabolised to DMPs, establishment of a sensitive specific urinary biomarker for dimethoate is not possible. Dimethoate will be included in the DMPs (see above for Cat B)</p> <p>After fipronil exposure, the major metabolite, fipronil sulfone, is rapidly formed. This metabolite is rather persistent and toxic in mammals. A method to measure fipronil sulfone in serum is available and seem to be the best biomarker for fipronil exposure.</p> <p>Recently, a method to measure hydroxyl-fipronil in urine was developed using rat urine but this method has not yet been applied on human samples.</p>	<p>Activities:</p> <ol style="list-style-type: none"> 1. If prioritised to include fipronil (Q2), urine will be the preferred matrix, allowing analyses of all the pesticides in the same samples. Thus, a method to measure its metabolite in human urine should be further developed and validated (WP9) 2. If prioritised to include POEA (Q2), the first step will be to collect available information on toxicokinetic i.e., absorption after different exposure routes, metabolism, and major urinary metabolites to evaluate if it is possible to establish a sensitive and reliable biomarker method (WP9).
2. What are the current exposure levels of the EU general population to the prioritised pesticides?	Cat B (pyrethroids, chlorpyrifos, and glyphosate)	<p>HBM studies including these substances have been performed in some EU countries but not EU-wide. The studies indicate widespread exposure in the general population. The exposure to pyrethroids is expected to be increasing as they replace organophosphates (OPs) in biocidal products and to some degree also as insecticides in agriculture.</p> <p>Children have higher food intake per kg body weight leading to higher exposure levels from pesticide residues in food as also confirmed in previous HBM-studies</p>	<p>Gaps: Few studies have been performed after 2010 and data are lacking for many EU countries. More data are needed to evaluate differences between countries and population groups, time trends, and age-related differences in exposure.</p> <p>Activities:</p> <ol style="list-style-type: none"> 1. Collecting, comparison, and evaluation of existing biomonitoring data in the EU and integration into IPCHEM (Y4) (WP8/WP10) 2. Further identify and prioritise knowledge and data gaps and related research needs (Y4) (WP4) 3. Planning and analysing supplementary urine samples from the alignment studies preferentially from children and from studies with available information on dietary habits and/or residential use of pesticides (Y4-Y5) (WP8) 3. Data-analyses of time-trends and differences between countries and population groups, including identification of subpopulations with highest exposure levels (Y4-Y5) (WP10). 4. Data analysis to identify differences between population groups related to e.g., age, dietary habits, residence near agricultural pesticide applications, and indoor residential use, occupational exposure (Y4-Y5) (WP10)

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Policy question	Substance	Available knowledge	Knowledge gaps and activities needed
	Cat C (dimethoate, fipronil, and POE-tallow amine (POEA))	<p>Studies from the US reported very low detection frequencies (< 1%) for dimethoate and omethoate, because they are rapidly metabolised to unspecific dimethyl phosphates (DMPs). Urinary DMPs and diethyl phosphates (DEPs) have been included in many studies as biomarker for the total OP exposure. Thus, including DMPs and DEPs will allow assessment of the total OP exposure (including dimethoate and chlorpyrifos) and comparison with previous studies.</p> <p>A recent case of fipronil misuse caused large scale contamination of chicken eggs but otherwise fipronil is seldom detected in commodities at the EU market. Fipronil is approved as biocide and for veterinary use but no longer for agricultural use in EU.</p> <p>There is reliable evidence that POEA increase the toxicity of some glyphosate formulations. Although POEA was recently banned in the EU, exposure from residues in food items (imported or due to contaminated soils) is very likely but there is no monitoring data from commodities or other potential human exposure sources to underpin the relevance of HBM.</p>	<p>Gaps: No EU HBM studies have included urine concentrations of dimethoate or its specific metabolite omethoate. There is no HBM data from EU on fipronil or POEA.</p> <p>Activities:</p> <ol style="list-style-type: none"> 1. Consider to include DMPs and DEPs in the analyses of supplementary urine samples from the alignment studies, as suggested above for the cat. B substances, to allow assessment of the total OP exposure (including dimethoate and chlorpyrifos) and comparison with previous studies (WP8). 2. Prior to method development for POEA and fipronil (see Q1) it should be considered whether to prioritise to monitor these substances in human matrices (preferentially urine) at present (WP4). 3. If prioritised, methods for analysing POEA and fipronil in urine has to be developed (WP9) – see Q2 for Cat C substances below, and samples from the alignment studies or from targeted studies will be analysed for fipronil and/or POEA (WP8)
3. What are the main dietary sources of exposure across the member states?	Cat B and C (all substances)	<p>Residues in the diet is the main continuous exposure source for pesticides in the general population. Pesticide residues in food is measured under coordinated control programmes (EUCP) and the national control programmes (NP). The coordinated multiannual control programme for 2018, 2019, and 2020 (Regulation (EU) 2017/660) includes many of the HBM4EU selected pesticides (i.e., 12 different pyrethroids, chlorpyrifos/chlorpyrifos methyl, dimethoate, glyphosate and fipronil). These data are collected and stored by EFSA (European Food Safety Authority 2017, 2018).</p> <p>Human breast milk samples are not included in the control programmes. Chlorpyrifos and pyrethroids have been found in breast milk samples from other countries (e.g., USA, India, Brazil and Colombia) sometimes in concentrations exceeding the MRL of 0.01 mg/kg for food for infants and young children (Directive 2006/141/EC). Only six samples from EU (Spain) have been analysed. Methods to analyse pyrethroids and chlorpyrifos in human milk samples are available.</p>	<p>Activities:</p> <ol style="list-style-type: none"> 1. Continue to analyse/model HBM data in relation to monitoring data on residues in food samples (EUCP, EFSA) to 1) compare and complement exposure assessment performed by EFSA and 2) identify the major dietary exposure sources across member states (Y4-Y5) (WP12) 2. Consider if possible, to perform a pilot study analysing selected pyrethroids and chlorpyrifos (parent compounds) in existing bio-banked milks samples (WP9, WP8).

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Policy question	Substance	Available knowledge	Knowledge gaps and activities needed
4. What are other sources and pathways of exposure?	Cat B and C (all substances)	<p>Living near agricultural areas where pesticides are applied may enhance the exposure level to pesticides due to drifting, as demonstrated in studies from the US. No HBM data from EU are currently available.</p> <p>A targeted survey including families (children and adults) living close to pesticide treated agricultural areas (3-5 countries) using a new developed multi-target screening of multiple pesticides in urine samples is planned in WP15 and WP16 (Survey on Pesticide Mixtures in Europe, SPECIMEn)</p> <p>Indoor use of pyrethroids and/or fipronil as biocides has been shown in studies from the US to contribute markedly to the exposure level – especially among children.</p>	<p>Activities:</p> <p>1. Analysing the urine samples from the WP15/16 survey using the above-mentioned methods, will allow quantification of these pesticide metabolites and subsequent data analyses to compare the levels with those obtained from the alignment studies (WP10) and comparison with the result obtained by the multi-target screening method in WP15/16 ((Y4-Y5) (WP8/WP9/WP10)</p> <p>Data gap: Biocidal use of pyrethroids might be increasing in the EU but there is no HBM studies investigating this exposure situation.</p> <p>Activities:</p> <p>1. A targeted study, focusing on children living in homes with repeated residential use of biocides would be highly relevant, e.g., with urine sampling before and fixed time points after treatment (WP8).</p> <p>2. If a targeted study is not prioritised it may be possible to get some information by analysing HBM data from the alignment studies (including additional analyses of urine samples from children) in relation to questionnaire information on residential use, if such data are available (preferentially with information on time interval between sampling and treatment). Data on authorisation and sale of biocidal products might also be included in data analysis of HBM data from the alignment studies to investigate exposure differences between member states (WP10).</p>

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Policy question	Substance	Available knowledge	Knowledge gaps and activities needed
5. What are exposure levels among occupationally exposed workers?	Cat B and C (all substances)	<p>Occupational exposure to agricultural workers who mix and/or apply pesticides onto crops can be substantial, with dermal exposure considered the most important pathway, although inhalation may also be important. Also, workers handling crops/plant after treatment have enhanced exposure and, since many young women in fertile age groups, are employed in agriculture/horticulture/floriculture they constitute a special risk group.</p> <p>Further, workers employed in companies applying biocides (pyrethroids and/or fipronil) in dwellings and institutions might have high dermal and inhalation exposure.</p>	<p>Data gap:</p> <p>There is no HBM data from EU covering occupational exposure of the selected pesticides. Investigating occupational exposure levels is important to identify high exposure groups.</p> <p>Activities:</p> <p>A targeted study addressing occupational exposure levels is highly relevant (WP8) as this Q cannot be answered based on the alignment study.</p> <p>The WP15/WP16 mixture survey will provide data on exposure profile/level among residents (children and mothers) close to agricultural fields (orchards) in five EU-countries.</p> <p>Including urine sampling also from agricultural workers who mix and/or apply the pesticides in this survey would allow additional information on occupational exposure (WP15, WP16, WP8)</p>
6. Are the exposure levels of health-relevance/concern for vulnerable groups or high exposure population groups?		<p>Most of the prioritised pesticides are neurotoxicants (OPs, pyrethroids, fipronil) and some also have ED or genotoxic/carcinogenic properties.</p> <p>The main health concerns are adverse effects on neurodevelopment and/or endocrine disturbances affecting reproduction, metabolism etc.</p>	<p>Activities:</p> <ol style="list-style-type: none"> 1. Combining and analysing HBM data (, e.g. from birth cohort studies) with relevant health outcomes – if possible using meta-analysis (Y4-Y5) (WP13) 2. Identify/suggest adverse outcome pathways (AOPs) for and effect biomarkers for relevant health outcomes (including neurodevelopment and endocrine disrupting effects) (Y4-Y5) (WP13/WP14). 3. Identify/suggest relevant effect biomarkers (WP14) 4. Comparison of exposure levels (based on HBM data) with toxicologically derived guidance values (ADI values) and findings on associations with health outcomes in epidemiological studies (Y4-Y5) (WP5, WP13)
7. How can cumulative risks of pesticide mixtures on health outcomes be assessed and integrated in regulation?		<p>Assumed additivity within the pesticide groups (similar mode of action; e.g. pyrethroids) but also across groups (similar adverse effects; e.g., neurotoxicity of pyrethroids and OPs)</p>	<p>Input from WP15 and activities mentioned for Q6</p>

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Policy question	Substance	Available knowledge	Knowledge gaps and activities needed
8. Is it possible to establish EU wide accepted health-based guidance values for the pesticides, preferably taking potential mixture effects and evidence from epidemiological studies into account?			See activities for Q6 Input from WP5
9. How can HBM data from HBM4EU feed into prioritisation of the pesticides for risk assessments and regulatory decision-making?			Input from WP5

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Annex 1: HBM data on pyrethroids (3-PBA), OPs (DAPs), chlorpyrifos/chlorpyrifos-methyl (TCPY) and glyphosate

Table 20-3: HBM data on pyrethroid exposure based on urinary concentrations of the generic pyrethroid metabolite 3-PBA. The values represent volume-based concentrations ($\mu\text{g/L}$) in spot urine samples unless otherwise stated.

Study	Population	Sampling year	N	LOD	%>LOD	GM	50th pct (median)	95 th pct	Remark	Ref
Europe										
PELAGIE, France	Pregnant Women Children, 6 yrs	02-06	205 284	0.008	30.2 63.7	- -	<LOD 0.018	- -	first trim, first-morning-voids	Viel et al. (2015)
Efe, France	Pregnant women, at delivery	2011	1077	0.004	99.7	0.36	0.36	1.89	Analysed in Canada	Dereumeaux et al. (2016)
GerES, Germany,	Children 3-14 yrs	03-06	598	0.1	98	-	0.43	3.80		Schulz et al. (2009)
Poland (North)	All <18 yrs >18 yrs	12	374 184 190	0.1	82.4 - -	0.26 0.29 0.23	0.25 - -	1.24 - -		Wielgomas and Piskunowicz (2013)
Poland (Lodz)	Adult men, age < 45 y	2008-11	195	0.1		0.17	0.16	0.50		Radwan et al. (2015)
Poland, Gdansk)	Genral pop, 5-77 y	2010-11	132	0.1	80	0.26	0.25	1.15	First morning voids	Wielgomas et al. (2013)
Spain	Children 6-11 yrs	10		0.8	23	-	<LOD	12.33*	* $\mu\text{g/g}$ creatinine	Roca et al. (2014)
OCC, Denmark	Pregnant Women	10-12	858	0.03	94.3	0.22	0.20	2.18	Fasting, GW 28	Dalsager et al. (2018)
Greenhouse Cohort Children, Denmark	Children 10-16 yrs Children 10-16 yrs	11-13	143 128	0.03	100 100	0.66 0.51	0.56 0.49	8.90 8.98	Non-fasting Fasting	unpublished
Greenhouse Cohort Children, Denmark	6-11 yrs	07-08	173	0.8	41.0	0.66	<LOD	4.11	first-morning-voids	Andersen et al. (2012)

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Study	Population	Sampling year	N	LOD	%>LOD	GM	50th pct (median)	95 th pct	Remark	Ref
America										
NHANES, USA	Children 6-11 yrs 20-59 yrs	09-10	383 1296	0.1		0.55 0.42	0.48 0.39	8.51 6.95		CDC (2015)
NYC HANES, USA	>20 yrs	04	1452	0.64	58.5	-	0.76	5.23		McKelvey et al. (2013)
SUPERB, USA	Children 2-8 yrs 18-57 yrs	07-09	83 64	0.75	60 90		1.56 1.58	4.69 9.44	Residential use	Trunnelle et al. (2014b)
MICASA, USA	Children 2-8 yrs Mothers 23-52 yrs	09	103 105	0.1	78 82	1.11 1.17	1.93 1.63	7.36 13.29	Farm worker families	Trunnelle et al. (2014a)
CHAMOCOS, USA	Pregnant Women	99-01	481	0.1	27	-	<LOD	1.1	Agricultural area, Second trim, GW 26	Castorina et al. (2010)
Mt. Sinai, New York, USA	Pregnant women	98-01	307			-	18.3	126.9*	Third trim, *90th pct Residential use	Berkowitz et al. (2003)
CHMS, Canada	Children 6-11 yrs All, 6-79 yrs	07-09	1032 5604	0.01	99.3 99.4	0.25	0.20 0.23			Oulhote and Bouchard (2013); Ye et al. (2015)
ELEMENT, Mexico	Pregnant women	97-01	187	0.25	56	0.26	<LOD	0.85	third trimester	Watkins et al. (2016)
Caribbean	Pregnant women	08-11	297	0.01	100	0.54	-	3.51	third trimester	Dewailly et al. (2014)
PROTECT, Puerto Rico	Pregnant Women	10-12	54	0.1		0.2	<LOD	2.3	second trimester	Lewis et al. (2014)
Asia										
Japan	Pregnant Women	09-11	231	0.02	97.8	0.33	0.35	-	GW 10-12	Zhang et al. (2013)
China	Pregnant Women	10-12	322	0.1	82	0.37	0.50	2.6		Ding et al. (2015)

GM: geometric mean; GW: gestational week

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Table 20-4: HBM data on OPs. Urinary concentrations of total dialkyl phosphate metabolites (Σ DAP; molar sum of DEPs and DMPs). The values represent volume based concentrations (nmol/L) in spot urine samples unless otherwise stated.

Study	Population	Sampling Year	N	GM	50th pct (median)	95 th pct	Remark	Ref.
Europe								
OCC, Denmark	Pregnant women	10-12	564	58.7	56.5	253	Fasting, GW 28	Dalsager et al. (2018)
Greenhouse Cohort Children,	Children 10-16 yrs	11-13	141	89.7	85.6	506		Andersen et al. (in publication)
Greenhouse Cohort Children,	Children 6-11 yrs	07-08	172	160.4	153.7	1252	First-morning-voids	Andersen et al. (2012)
DEMOCOPHES DK-part, Denmark	Women 31-52 yrs Children 6-11 yrs	11	145 144	84.8 111	92.3 106		First-morning-voids	Mørck et al. (2016)
PELAGIE	Pregnant women	02-06	254		38.8		First-morning-voids	Debost-Legrand et al. (2016)
Generation R, Holland	Pregnant women	04-06	100	183	200	659	GW 20	Ye et al. (2008)
MoBa, Norway	Pregnant women	99-04	10	87*			10 pools, each consisting of pooled urine from 11 women * Calculated from $\mu\text{g/L}$	Ye et al. (2009)
Greece, Crete	Adults	08-09	86	-	15	-	Agricultural area	Kokkinaki et al. (2014)
America								
NHANES, USA	Children 8-15 yrs	00-04	1139	68.3				Bouchard et al. (2010)
HOME, Ohio, USA	Pregnant women	03-06	327	73.7*	96.7*		*nmol/g creatinine, two spot urine samples during preg,	Donauer et al. (2016)
NYC HANES, USA	Adults >20 yrs	04	876	-	114.9	1321.8		McKelvey et al. (2013)

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Study	Population	Sampling Year	N	GM	50th pct (median)	95 th pct	Remark	Ref.
Mount Sinai, USA	Pregnant women	98-02	342	75.5*	77.9*	894.7*	*nmol/g creatinine, residential use	Harley et al. (2016)
CHAMACOS, U.S.	Pregnant women Children 5 yrs	99-00 04-05	348 320	109 92.6	-	-		Marks et al. (2010)
MIREC, Canada	Pregnant women	08-11	1884	78	78	538	First trim	Sokoloff et al. (2016)
CHMS, Canada	Children 6-11 yrs All, 6-79 yrs	07-09	1035 5604	- 76.7	99.2 71.4	-		Oulhote and Bouchard (2013); Ye et al. (2015)

GM: geometric mean; GW: gestational week

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Table 20-5: HBM data on chlorpyrifos/chlorpyrifos-methyl. Urinary concentrations of the specific metabolite TCPY. The values represent volume-based concentrations ($\mu\text{g/L}$) in spot urine samples unless otherwise stated.

Study	population	Sampling year	LOD	%>LOD	N	GM	50th pct (median)	95 th pct	Remark	Ref
Europe										
OCC, Denmark	Pregnant women	10-12	0.3	93.2	858	1.67	1.74	8.15	Fasting, GW 28	Dalsager et al. (2018)
Greenhouse Cohort Children	Children 10-16 yrs	11-13	0.3	95.8 94.5	143 128	1.42 1.52	1.43 1.55	6.05 7.31	Non-fasting Fasting	Andersen et al. (in publication)
MoBa, Norway	Pregnant women	99-04			10*	0.99			second trim, *pooled samples	Ye et al. (2009)
Generation R, The Netherlands	Pregnant women	04-06	0.15	100	100	1.2	1.2	6.4	> GW 20	Ye et al. (2008)
Spain	Children 6-11 yrs	10	0.80	86	125	3.36*	3.40*	12.97*	* $\mu\text{g/g}$ creatinine	Roca et al. (2014)
America										
NHANES, USA	Children 6-11 yrs Adults 20-59 yrs	09-10	0.1		386 1309	1.12 0.71	1.46 0.97	5.81 4.18		CDC (2015)
CHAMOCOS, USA	pregnant women	99-01	0.3	81.9	481	-	3.2	17.9	Agricultural area, Second trim, GW 26	Castorina et al. (2010)
Mt. Sinai, USA	pregnant women	98-01			365	-	7.5	61.2*	Third trim, *90th perc Residential use	Berkowitz et al. (2003)
ELEMENT, Mexico City	pregnant women	97-05	0.1	>90	187	1.76	1.78	11.6	Third trim	Fortenberry et al. (2014)
Puerto Rico	Pregnant women	10-12	0.1	86.2	54	0.4	0.5	2.0	4 samples per women	Lewis et al. (2015)
Australia	Children 2.5-6 yrs	03-06		92.2	115	-	12.5*	71.1*	* $\mu\text{g/g}$ creatinine	Babina et al. (2012)

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Study	population	Sampling year	LOD	%>LOD	N	GM	50th pct (median)	95 th pct	Remark	Ref
Asia										
China	Children 3-6 yrs	2014		44.1	406	0.92*	0.63*	22.9*	*µg/g creatinine	Wang et al. (2016)

GM: geometric mean; GW: gestational week

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Table 20-6: HBM data on glyphosate and AMPA. Urinary concentrations ($\mu\text{g/L}$) in various European countries

Reference	Country	Period	Population	N	Definition of average	Value average**	Calculation high values	High value**
3e Flemish Center of Expertise on Environment and Health (Steunpunt M&G)	Belgium (Flanders)	2013-2014	Adults (50-65 years), general population	269	GM (95% CI)	Gly: <LOQ AMPA: 0,109 (0,098-0,120)a	P90 (95% BI)	Gly: 0,230a AMPA: 0,344 (0,135-0,553)a
Paulussen, 2013	Belgium (Flanders)	2012-2013	Teenagers (14-15 years), general population	11	P50	Gly: 0,30 AMPA: 0,33	P75	Gly: 0,40 AMPA: 0,60
BUND, 2013 (Hoppe 2013)	Belgium	2013		11	average	Gly: 0,18 AMPA: 0,29	max	Gly: 0,57 AMPA: 1,26
	Netherlands	2013		8	average	Gly: 0,34 AMPA: 0,25	max	Gly: 1,02 AMPA: 0,64
	France	2013		10	average	Gly: 0,12 AMPA: 0,14	max	Gly: 0,23 AMPA: 0,41
	Germany	2013		10	average	Gly: 0,25 AMPA: 0,23	max	Gly: 0,49 AMPA: 0,70
	Great-Britain	2013		10	average	Gly: 0,47 AMPA: 0,23	max	Gly: 1,64 AMPA: 0,56
	Switzerland	2013		12	average	Gly: 0,09 AMPA: 0,08	max	Gly: 0,16 AMPA: 0,08
	Spain	2013		10	average	Gly: 0,12 AMPA: 0,17	max	Gly: 0,22 AMPA: 0,82
Danish part of DEMOCHOPHES, Knudsen et al. (2017)	Denmark	2011	Children 6-11 yrs	14	Mean	Gly: 1.96	max	Gly: 3.31

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Reference	Country	Period	Population	N	Definition of average	Value average**	Calculation high values	High value**
	Denmark	2011	mothers	13	Mean	Gly: 1.18	max	Gly: 3.22
Conrad et al. (2017)	Germany (Greifswald)	2011	20-29 yrs	40	P50	Gly: <LOQ AMPA: <LOQ	max	Gly: 0.51 AMPA: 0.65
	Germany (Greifswald)	2012	20-29 yrs	40	P50	Gly: 0.11 AMPA: 0.12	max	Gly: 0.63 AMPA: 0.66
	Germany (Greifswald)	2013	20-29 yrs	40	P50	Gly: 0.11 AMPA: <LOQ	max	Gly: 2.80 AMPA: 1.88
	Germany (Greifswald)	2014	20-29 yrs	40	P50	Gly: <LOQ AMPA: <LOQ	max	Gly: 1.78 AMPA: 0.97
	Germany (Greifswald)	2015	20-29 yrs	40	P50	Gly: <LOQ AMPA: <LOQ	max	Gly: 0.57 AMPA: 0.41

^acorrected for age, sex, smoking and urine density

** Gly = glyphosate

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Annex 2: HBM4EU-suggested pesticide biomarkers/metabolites and reference to established analytical methods

Pesticide group	Metabolite/biomarker (abbreviation)	Cas no	Parent pesticide/compound	Matrix	Analytical methods, reported LOD/LOQ in ng/ml (Reference)	Status
Pyrethroid	3-phenoxybenzoic acid (3-PBA)	3739-38-6	Common metabolite of most pyrethroids, e.g.: cypermethrin, deltamethrin, permethrin, lambda-cyhalothrin, d-phenothrin, tau-fluvalinate, esfenvalerate, fenpropathrin, (not cyfluthrin or bifenthrin)	Urine	LC-MS/MS, 0.03 (Davis et al. 2013); UPLC-MS/MS, 0.008 (Viel et al. 2015); GC-MS, 0.1 (Becker et al. 2006; Wielgomas and Piskunowicz 2013); LC-MS/MS, 0.8 (Roca et al. 2014); GC-MS, 0.1 (0.5 nM) (Bevan et al. 2013)	OK
Pyrethroid	4-fluoro-3-phenoxybenzoic acid (F-3PBA)	77279-89-1	Cyfluthrin	Urine	LC-MS/MS, 0.03 (Davis et al. 2013); UPLC-MS/MS, 0.003 (Viel et al. 2015); GC-MS, 0.1 (Becker et al. 2006; Wielgomas and Piskunowicz 2013); LC-MS/MS, 0.2 (Roca et al. 2014); GC-MS, 0.1 (0.5 nM) (Bevan et al. 2013)	OK
Pyrethroid	cis-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid (Cis-DCCA)	55701-05-8	Cis-permethrin, cis-cypermethrin, cis-cyfluthrin	Urine	LC-MS/MS, 0.4 (Davis et al. 2013); GC-MS/MS, 0.07 (Viel et al. 2015); GC-MS, 0.1 (Becker et al. 2006; Wielgomas and Piskunowicz 2013); LC-MS/MS, 0.4 (Roca et al. 2014); GC-MS, 0.1 (0.5 nM) (Bevan et al. 2013)	OK
Pyrethroid	trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid (Trans-DCCA)	55701-05-6	Trans-permethrin, trans-cypermethrin, trans-cyfluthrin	Urine	LC-MS/MS, 0.4 (Davis et al. 2013); GC-MS/MS, 0.01 (Viel et al. 2015); GC-MS, 0.1 (Becker et al. 2006; Wielgomas and Piskunowicz 2013); LC-MS/MS, 0.4 (Roca et al. 2014); GC-MS, 0.1 (0.5 nM) (Bevan et al. 2013)	OK

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Pesticide group	Metabolite/biomarker (abbreviation)	Cas no	Parent pesticide/compound	Matrix	Analytical methods, reported LOD/LOQ in ng/ml (Reference)	Status
Pyrethroid	cis-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid (Cis-DBCA)	63597-73-9	Deltamethrin	Urine	LC-MS/MS, 0.4 (Davis et al. 2013); GC-MS/MS, 0.07 (Viel et al. 2015); GC-MS, 0.1 (Becker et al. 2006; Wielgomas and Piskunowicz 2013); LC-MS/MS, 0.8 (Roca et al. 2014); GC-MS, 0.1 (0.5 nM) (Bevan et al. 2013)	OK
Pyrethroid	4-chloro-alpha-isopropyl benzene acetic acid (CPBA)		Esfenvalerate, fenvalerate	Urine	GC-MS, 0.04(Tao et al. 2013)	Probably not relevant, likely low DF*
Pyrethroid	2-methyl-3-phenylbenzoic acid (MPA)	115363-11-6	Bifenthrin	Urine	GC_MS, 0.04(Tao et al. 2013)	?
Pyrethroid	3-(chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropane carboxylic acid (HCVA)		Lambda-cyhalothrin	Urine	GC-MS, 0.08(Tao et al. 2013)	?
Pyrethroid	Chlorotrifluorovinylcyclopropane carboxylic acid (ClF-3-CA)		Bifenthrin and cyhalothrin	Urine	GC_MS, 0.5 nM(Bevan et al. 2013)	Might be relevant?
Pyrethroid	Parent compounds		Permethrin, cypermethrin, cyhalothrin, deltamethrin	Human breast milk	GC-NCI-MS-MS analysis, 2.8-1100 pg/g lipid weight (Corcellas et al. 2012).	Pilot study?
Pyrethroid synergist (co-formulant)	Piperonyl butoxide (PBO)		Piperonyl butoxide	Urine	UHPLC-QqQ-MS/MS, 0.047/0155.(Cazorla-Reyes et al. 2011) Below LOD in all urine samples analyzed by LC-MS/MS at SDU (unpublished).	?
Organophosphate	3,5,6-trichloro-2-pyridinol (TCPY)		Chlorpyrifos and chlorpyrifos-methyl	Urine	LC-MS/MS, 0.1 (Davis et al. 2013); GC-MS/MS, 0.15 (Ye et al. 2008); UPLC- HRMS, 0.8 (Llop et al. 2017b)	OK

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Pesticide group	Metabolite/biomarker (abbreviation)	Cas no	Parent pesticide/compound	Matrix	Analytical methods, reported LOD/LOQ in ng/ml (Reference)	Status
Organophosphate	Dimethoate and omethoate		Dimethoate	Urine	LC-MS/MS, 0.03 and 0.05 (Llop et al. 2017a)	Not sensitive - out
Organophosphate	Diethyl phosphate (DEP), diethyl thiophosphate (DEDTP), diethyl dithiophosphate (DEDTP)		Unspecific metabolite of ethyl-organophosphates e.g., chlorpyrifos, diazinon, ethion, coumaphos, terbufos	Urine	LC-MS/MS, 0.1-0.5 (McKelvey et al. 2013); GC-MS/MS, 0.65, 0.59, 0.05 nM (Ye et al. 2008); UPLC- HRMS, 3.2-10 (Llop et al. 2017b)	OK
Organophosphate	Dimethyl phosphate (DMP), dimethyl thiophosphate (DMTP), dimethyl dithiophosphate (DMDTP)		Unspecific metabolite of methyl-organophosphates, e.g., dimethoate, chlorpyrifos-methyl, azinphos-methyl, malathion, fenthion, phosmet, naled	Urine	LC-MS/MS, 0.1-0.5 (McKelvey et al. 2013); GC-MS/MS, 0.79, 0.70, 0.63 nM (Ye et al. 2008); UPLC- HRMS, 1.6 (Llop et al. 2017b)	OK
Glyphosate	Glyphosate (Gly) and aminomethylphosphonic acid (AMPA)		Glyphosate and AMPA	Urine	GC-MS/MS, 0.1(Conrad et al. 2017); LC-MS/MS, 0.1 (Parvez et al. 2018)	OK
Co-formulant with Glyphosate	?		Polyethoxylated tallowamine (POEA)	?	?	?
Fipronil	Hydroxyl-fipronil		Fipronil	Urine	LC-MS/MS, 0.4 (rat urine)(Vasylieva et al. 2017)	OK
Fipronil	Fipronil sulfone, fipronil desulfinyl		Fipronil	Serum/plasma or breast milk	LC/TOF-MS, 0.1 (McMahen et al. 2015)	?

*DF: detection frequency

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21 Prioritised substance group: Phthalates & Hexamoll® DINCH®

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21.1 Background information

21.1.1 Hazardous properties

Phthalates (or phthalate esters) and the non-phthalate substitute Hexamoll® DINCH® are a group of plasticizers with a production volume of millions of tons per year. Phthalates can cause a variety of adverse effects in humans and in laboratory animals of which the most prominent are the endocrine disrupting and reproductive effects (German HBM Commission 2011; US CPSC 2014c; Mariana et al., 2016; Radke et al., 2018). It has been shown that some phthalates, such as di(2-ethylhexyl) phthalate (DEHP), butylbenzyl phthalate (BBzP), di-n-butyl phthalate (DnBP), and di-iso-butyl phthalate (DiBP) induce the so-called phthalate syndrome, which covers different reproductive abnormalities in male offspring of rats exposed during pregnancy with the critical time window of gestation day 15-17. The effects are among others malformations of the testes, epididymides and Gubernaculum Testis, cryptorchism, hypospadias, reduced semen count and others caused by interference of the development of foetal Leydig cells, reduced or inhibited testicular testosterone production and reduced production of insulin-like 3 peptide hormone (German HBM Commission 2011; NRC 2008). Not all phthalates exhibit the reprotoxic and developmental effects described above and not all have the same endocrine disrupting potency. It seems that the molecular structure of the alkyl side chain is responsible for the exhibition of the effects. It appears that only those phthalates are toxic to the male development that have a linear ester side length of a total of 4-7 carbon atoms (e.g. DnBP) or that have a branched side chain of a total of 4-9 (e.g. DEHP, DiBP). Phthalates with less or more carbon atoms in the side chain do not appear to exhibit effects on the reproductive development on the male foetal rat (Furr et al., 2014; Kortenkamp and Koch, 2020; Li et al., 2019). The most potent representative is di-n-pentyl phthalate (DnPeP), followed by DEHP, DnBP, DiBP, BBzP, di-n-hexyl phthalate (DnHP) and dicyclohexyl phthalate (DCHP) with a comparable potency. Di-iso-nonyl phthalate (DiNP) has the lowest potency to act as an endocrine disruptor (German HBM Commission 2011; US CPSC 2014c). It must be assumed, that similar adverse effects are also caused in human, since the effects of the phthalate syndrome in rats have similarities with the observed testicular dysgenesis syndrome in humans (US CPSC 2014c; Hu et al., 2009; Skakkebaek et al., 2001). In addition, several epidemiological studies conjecture an association between phthalate exposure and overweight, insulin resistance, asthma, attention deficit/ hyperactivity disorder or other metabolic diseases and neurodevelopmental outcomes (Engel et al., 2010; Franken et al., 2017; Hatch et al., 2010; Radke et al., 2020; Radke et al., 2019; Robinson and Miller, 2015; Wang et al., 2015).

In terms of risk assessment, it is important to note that mixtures of the above mentioned phthalates have direct additive effects (Howdeshell et al., 2017), but also additive effects with other endocrine disrupting chemicals has been demonstrated, even though they function via a different mode of action (Rider et al., 2010). Due to increased knowledge of the endocrine disrupting effects of the above-mentioned phthalates, less harmful plasticisers became more important over the last decade including di(2-propylheptyl) phthalate (DPHP) and Hexamoll® DINCH®. DPHP due to its molecular structure is thought to have no anti-androgenic effects, but only limited data is available.

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Effects on the thyroid gland observed in a subchronic and a two-generation studies on rats were evaluated as most critical effects by different bodies (NICNAS 2003; BASF 2009b; German HBM Commission 2015; BASF, 1995b; Bhat et al., 2014). Signs of toxicity to the reproduction or development were not observed in the toxicity studies mentioned. Hexamoll® DINCH® was introduced into the market in 2002 as a substitute mainly for DEHP and DiNP. The currently available data suggests that Hexamoll® DINCH® has no reproductive effects and is not an endocrine disrupter, but nephrotoxic effects were observed in a subchronic and a two-generation feeding study in rats (BASF 2003b; BASF, 2002a). EFSA (2006) considered these effects relevant for the derivation of a TDI of 1 mg/kg bw/d (EFSA 2006). A study of women undergoing in vitro fertilisation treatment by Mínguez-Alarcón et al., 2016 showed suggestive negative associations between urinary MINCH concentrations and peak estradiol levels and numbers of total oocyte yields with stronger associations in older women compared to younger women (Minguez-Alarcon et al., 2016). In animal studies effects on the thyroid gland are observed but were considered as secondary effects associated with liver enzyme induction and therefore of limited relevance to humans (EFSA 2006).

21.1.2 Exposure characteristics

Phthalates, more specifically orthophthalates are the most commonly used plasticisers globally with an annual consumption of 8.4 million tons. Orthophthalates are made of alcohols with long alkyl chains and 1 million tons of orthophthalates are produced each year in Europe, which represent 80% of the plasticiser market. Depending on their molecular structure, they can be differentiated into low molecular weight orthophthalates (LMW) and high molecular weight (HMW) with different physico-chemical properties resulting in different applications. The latter include DEHP, DiNP, di-iso-decyl phthalate (DiDP) or di-2-propylheptyl phthalate (DPHP) of which the majority is used in flexible polyvinyl chloride (PVC) products such as flooring, wires and cables, sport equipment, toys, coated textiles, footwear, synthetic leather and others. DEHP is also used in PVC medical devices (Koch and Angerer, 2012). The LMW phthalates comprise DiBP, BBzP, DnBP, diethyl phthalate (DEP) and dimethyl phthalate (DMP) are more volatile and have plasticising and solvent-like properties. Therefore, they have various other applications in addition to PVC products such as gelling plasticizers, paints, dispersions, and adhesives, but also as solvents in insect repellents (DMP) and in cosmetics (DEP). DEP and DnBP are also used in enteric-coated tablets/capsules as enteric film-coating materials or matrix binder (Wittassek et al., 2011a). It is noteworthy that there is no coherent classification into LMW and HMW phthalates and different classifications have been used (European Plasticisers/Health Canada, 2011). Hexamoll® DINCH®, due to its low toxicity and low migration rate, is used in soft PVC-containing medical devices such as blood bags, in food contact materials, such as artificial wine corks, in sports equipment and textile coatings, in wallpaper, paints and inks, adhesives and in cosmetics and toys. In the latter, Hexamoll® DINCH® is thought to be the most used plasticiser alternative.

However, DnBP, DiBP, BBzP, DEHP, DMEP, DnPeP, DiPeP are generally not allowed to be placed on the EU-market, when used as individual substances or in mixtures for supply to the general public when concentration limits are equal to or exceed 0,3%. In addition, DEHP, DnBP, BBzP, DEHP, DnPeP, DiPeP and DHNUP are prohibited for use in cosmetics in the European Union. Nevertheless, consumer articles (e.g. from Asia or USA) can contain phthalates since there is no such strict restriction for the use of phthalates in consumer articles up to now.

Since phthalates are not chemically bound to the (plastic) materials, they can leach, migrate or evaporate into indoor air and atmosphere, foodstuff or other materials and so are of ubiquitous presence in the environment (Heudorf et al., 2007). Therefore, plasticizers can be taken up by ingestion, inhalation and dermal contact. Whereas for HMW phthalates the main source of

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exposure is via food originating from contamination, e.g. via food contact materials (ECHA 2012; Wittassek et al., 2011b), especially for DEHP but also for DiNP, the exposure to LWM phthalates from food contribute to the overall exposure to a minor degree (German HBM Commission 2011). Inhalation of indoor air, exposure via ingestion of house dust by children and dermal contact with articles and dust can also be sources of exposure, especially for the short chain phthalates. Fromme et al., 2013b found significant correlations between phthalate concentrations in dust samples and urinary levels of DnBP, BzBP, but also for DEHP (Fromme et al., 2013). In the German Environmental Survey on children and adolescents conducted in 2014-2017 (GerES V) high phthalate concentrations in house dust were associated with the respective phthalate metabolite levels in urine. Indeed, 1.6- to 2.0-fold higher GMs of urinary metabolites of DEP, DMP, BBzP, and DiBP and 1.2- to 1.3-fold higher GMs of urinary DEHP, DiNP, and DiDP metabolites were observed in study participants for which also a high concentration of the respective substance had been found in house dust samples compared to those that had lower concentrations of these compounds in the house dust samples (Schwedler et al., 2020). In addition, medical treatment can lead to high exposure towards certain phthalates. For example, an exposure source for DEHP can be medical devices, such as tubes in blood transfusion. Long-term treatment with enteric-coated tablets/capsules can lead also to high exposure of DEP and DnBP (EMA 2014b; SCENIHR 2016).

Several human-biomonitoring studies in the EU, US and Asia were conducted, showing that the ubiquitous use of phthalates lead to a continuous internal exposure of the general public. Phthalate metabolites are being detected in a high percentage of the study population, sometimes present in each urine sample investigated (Berman et al., 2013; Choi et al., 2017; Den Hond et al., 2015; Haines et al., 2017; Koch et al., 2017; Schwedler et al., 2020; Ye et al., 2008; Zota et al., 2014). Comparison of exposure estimates between studies from the DEMOCOPHES project revealed a clear age difference. Levels of metabolites were in general higher in children than in mothers, which is confirmed also by other studies (ECHA 2016; Becker et al., 2009; Frederiksen et al., 2013; Geens et al., 2014; Hartmann et al., 2015). The relative metabolite levels differed among countries, with Swedish children having higher urinary MBzP levels than the European average, Slovak children having two times higher concentrations of DEHP metabolite levels than the European average and Polish children showed highest levels of MnBP and MiBP. In Spain, average MEP levels were six times higher than the European average. However, exceedance of health-based guidance values, in particular HBM-I values and BE values, were only reported for few cases, i.e. for DEHP metabolites in mothers and children (Den Hond et al., 2015). Furthermore, results of some of the above-mentioned studies also suggest, that exposure to high levels of one phthalate metabolite is positively correlated with high exposure of one of the other phthalate metabolite (ECHA 2016; Becker et al., 2009; Frederiksen et al., 2013). However, this cannot be confirmed by results of the DEMOCOPHES study (Den Hond et al., 2015).

In the Annex XV Restriction Report on four phthalates of ECHA (2016) risk characterisation ratios (RCRs) for the health of the general public were calculated based on DEMOCOPHES data revealing that in 13 out of 15 Member States of the EU RCRs for combined 95th percentile exposure for DEHP, DnBP, BBzP and DiBP are at or above 1 for children (ECHA 2016). This stresses the fact, that cumulative risk assessment is crucial to accurately determine the hazards originating from phthalates exposure. Since endocrine active phthalates can act in a dose additive manner and humans simultaneously are exposed to multiple phthalates, cumulative exposure to phthalates might exceed health-based guidance values and therefore pose a risk to the public health (Apel et al., 2020; Chang et al., 2017; S eborg et al., 2012).

Nonetheless, phthalate use in the industry and in the consumer environment has changed dramatically during the past decade due to regulatory restrictions. Recently, Koch et al., investigated the time trend of phthalates exposure using urinary samples from the German

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Environment Specimen Bans (ESB) regularly taken in the time frame of 1988 until 2015. They showed that the exposure to old, well-known and restricted phthalates (DEHP, DnBP, BBzP) has decreased. When comparing DEMOCOPHES data to older studies, a significant decline in exposure was also seen for Germany and Denmark (ECHA 2016). Also other European countries have reported a decrease in phthalate metabolite levels (Frederiksen et al., 2020; Gyllenhammar et al., 2017; Koch et al., 2017; Schoeters et al., 2017). Exposure to the metabolites of the substitutes Hexamoll® DINCH® has increased in Germany and in Sweden (Frederiksen et al., 2020; Kasper-Sonnenberg et al., 2019). The European population is also significantly exposed to newer phthalates like DiNP and DPHP and it can be assumed that the European population will still be exposed to restricted phthalates in the future as recent biomonitoring studies indicate, e.g. due to long lifetimes of articles, recycled PVC and from remaining authorised uses or uses that are not restricted (Schütze et al., 2015; Shu et al., 2018).

21.1.3 Technical aspects

In the course of Human Biomonitoring of phthalates, the concentration of the degradation products (metabolites) are commonly analysed in urine due to its non-persistent nature, since urinary concentrations of the compounds and their metabolites are usually higher than in blood. After exposure, phthalates rapidly metabolised and are completely excreted within 24 hours (Wittassek et al., 2011a). Therefore, it is important to have comprehensive knowledge on the metabolism of the respective compound. LMW phthalates are generally determined via their primary monoester metabolites, whereas HMW phthalates are determined via their oxidised metabolites. The longer the alkyl side chains are the stronger is the oxidative modification of the monoester. Therefore, the LMW phthalates are excreted mainly as their monoesters and to a lesser extent as their oxidative metabolites. The HMW are excreted mainly as their oxidative metabolites and to a lesser extent as their simple monoesters. For some monoesters, e.g. MnBP, MiBP, MEHP and MiNP an internal and external contamination control is warranted. Due to the omnipresence of phthalates and usage in laboratory equipment external contamination of the sample with the parent compound or their monoester can occur. Biodegradation can lead to the contamination of the samples with monoesters, since they cannot be distinguished from the monoester that indicate the body burden. HMW phthalates as DiNP and DiDP are challenging due to their presence in different isomers. Separation of the isomers is difficult and experience is needed to identify the various isomers in the chromatogram.

For most of the phthalates discussed here, a solid mass spectrometry method exist. DPHP metabolites can be determined separately using a GC-MS method. Hexamoll® DINCH® metabolites can be measured using a LC-MS/MS but an external quality assessment scheme is currently not available. No or insufficient methods exist for DiPeP, DMEP and DHNUP. For more information please see here: <https://www.hbm4eu.eu/deliverables/>.

21.1.4 Policy relevance

DEHP, DnBP, DiBP, BBzP, DnPeP, DiPeP, DHNUP, DnHP and DMEP are classified as reproductive toxicants category 1B under Annex VI to the Classification, Labelling and Packaging (CLP) regulation (EC 1272/2008). Due to their reprotoxic properties and additionally for DEHP, BBzP, DnBP, DiBP and dicyclohexyl phthalate (DCHP) since 2017 and 2018, respectively due to their endocrine disrupting properties, these substances have been identified as substances of very high concern (SVHC) and therefore included in the candidate list for the inclusion in Annex XIV of the REACH regulation (Annex XIV of REACH EC 1907/2006). Four of the nine above mentioned phthalates are already subject to authorisation, namely DEHP, BBzP, DiBP and DnBP. Since February 2015 they must not be used within the European Union without authorisation. Applications for authorisation were submitted for DEHP and DnBP only. There are Commission

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decisions on some authorisations, others are currently under evaluation. However, imported goods do not come under the authorisation requirement. Since June 2017, three other phthalates are included in the Authorisation List: DiPeP, DMEP and DnPeP with a sunset date of July 2020.

The current restrictions under REACH also cover some phthalates to a certain extent. Reprotoxic substances, such as DEHP, BBzP, DnBP, DIBP, DnPeP, DiPeP, DCHP, DHNUP, DnHP and DMEP are generally not allowed to be placed on the market, in the EU as individual substances or in mixtures for supply to the general public when concentration limits are equal or exceed 0,3%. Furthermore, the use of DEHP, DnBP, DiBP and BBzP is restricted in plasticised materials of all toys and childcare articles with a concentration limit of 0.1% by entry 51 of Annex XVII to REACH. In addition, DiNP, di-*n*-octyl phthalate (DnOP), DiDP are restricted for all children's toys and childcare articles that can be placed in children's mouth with a concentration limit of 0.1% by entry 52 of Annex XVII to REACH. On July 2020, a further restriction on DEHP, DnBP, DiBP, and BBzP will take affect, which comprises that the above-mentioned phthaltes are restricted to a concentration equal to or below 0.1% by weight individually or in any combination in any plasticised material in articles used by consumers or in indoor areas (EC 2018).

For Hexamoll® DINCH® an analysis of the most appropriate risk management option (RMOA, January 2016) was conducted by the French Agency for food, environmental and occupational health & safety (ANSES). As a result, the suspicion of reprotoxicity and endocrine disrupting properties could not be confirmed. The effect on the thyroid gland observed in rats after exposition of Hexamoll® DINCH® cannot be generally applied to humans due to the higher susceptibility of the thyroid tissue to contaminants in rats and the relatively high doses used. The possible carcinogenicity in humans was negated. Furthermore, impairment of the environment was also negated, due to the production and use patterns. As a consequence, currently no further risk mitigation measures are necessary (ECHA 2016).

In addition to the REACH legislation, there is also a product-specific legislation which regulates certain phthalates, i.e. the Cosmetic Products' Regulation (EC/1223/2009) and the regulation on plastic materials and articles intended to come into contact with food (EC 1935/2004 and Directives 80/590/ECC & 89/109/ECC), more specific the Regulation for Plastics Implementation Measure (10/2011/EC).

In the Cosmetic Products' Regulation, Article 15 outlines the prohibition of CMR substances in cosmetic products. Furthermore, Annex II lists the substances that are prohibited for use in cosmetics. These include the following: DEHP, DnBP, BBzP, DMEP, DnPeP, DiPeP and DHNUP. In Annex I (Union List) of the regulation on plastic materials and articles intended to come into contact with food, all substances are listed, which are authorised for the use as starting material, excipient or additive for plastic layers in plastic materials and articles. Each substance must not exceed its specific migration limit (SML). The following phthalates and phthalate substitutes are authorised for use as excipient or additive: DEHP with a SML of 1.5 mg/kg foodstuff, BBzP with an SML of 30 mg/kg foodstuff, DnBP with a SML of 0.3 mg/kg foodstuff and DiNP, DiDP and Hexamoll® DINCH®. Thereby apply different use restrictions. DnBP, BBzP, DEHP, DiNP and DiDP can only be used as plasticiser for articles which come in contact with fatless foodstuff and BBzP, DiNP and DiDP cannot be used in articles containing infant formulas. Furthermore, for DnBP, BBzP, DEHP, DiNP, DiDP and Hexamoll® DINCH® applies a group restriction, that is, the sum of these substances must not exceed an SML of 60 mg/kg foodstuff.

Annex I, Part II, Article 7.5 of the Medical Device Directive (93/42/EWG) states that medical devices containing phthalates classified as CMR must be labelled and if these are intended to be used for children, nursing mothers and pregnant women the manufacture must give a specific justification for this use.

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DEHP, BBzP, DnBP and DiBP must not be contained in homogenous materials above the concentration of 0.1% from July 2019 on according to the Restriction of Hazardous Substances Directive in electrical and electronic equipment RoHS2 (2011/65/EC). For medical devices and *in-vitro* diagnostic products this restriction takes effect in July 2021.

For some of the phthalates Human Biomonitoring assessment values, namely Biomonitoring equivalents (BE) or HBM I values, have been derived – these are concentrations of biomarkers (metabolites) in urine, which reflect an acceptable chronic exposure, since the basic assumption is an equilibrium between external exposure and internal burden (German HBM Commission 2014a; Angerer et al., 2011; Apel et al., 2017; Hays et al., 2008; Hays et al., 2007). BE values have been derived for the Category A phthalates and HBM-I and HBM-II values are available for DEHP, DPHP and Hexamoll® DINCH®. In the course of the work done within the HBM4EU project EU-wide consolidated Human Biomonitoring guidance values for the general population (HBM-GV_{GenPop}) and for workers (HBM-GV_{workers}) are derived (Apel et al., publication submitted).

21.1.5 Societal Concern

Phthalates are a well-known group of plasticisers and are widely used since the 1920s. Due to the endocrine disrupting properties, some phthalates have been assigned with labelling requirements and use restrictions already in the late 1990s. Since then phthalates as a group are of great societal concern due to their toxicity to reproduction and omnipresence in the biological matrices of humans. Greenpeace conducted several studies addressing phthalates in consumer products and the potential health effects emerging from its endocrine disrupting effects (Allsopp et al., 2006). Many websites inform the public worldwide about consumer products free of phthalates. Furthermore, efforts have been made to reduce the phthalate uses in cosmetics and toys beyond the scope of European regulation as in the US, Japan and China. In addition, industry already substituted many of the endocrine disrupting phthalates with less potent or no endocrine disrupting substances, such as Hexamoll® DINCH®. All phthalates discussed here, except DPHP and the substitute Hexamoll® DINCH® are included in the SIN list.

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21.2 Categorisation of substances

Table 21-1: Substances included in the substance group, listed according to availability of toxicology and human biomarker data, in category A, B, C, D or E substances based on the Prioritisation Strategy and criteria elaborated under WP4, Year 1 (Deliverable D4.3)

Cat.	Abbrev./ Acronym	Systematic name	CAS No.	Classification (CHL/Annex III entry)	REACH Regulation
A	DEHP	Di(2-ethylhexyl) phthalate	117-81-7	CLH: Repr. 1B	REACH Annex XIV; Annex XVII, Entry 51
A	BBzP	Butyl benzyl phthalate	85-68-7	CLH: Repr. 1B; Aquatic Acute1; Aquatic Chronic 1	REACH Annex XIV; Annex XVII, Entry 51
A	DnBP	Di-n-butyl phthalate	84-74-2	CLH: Repr. 1B; Aquatic Acute 1	REACH Annex XIV; Annex XVII, Entry 51
A	DiBP	Diisobutyl phthalate	84-69-5	CLH: Repr. 1B	REACH Annex XIV
A	DEP	Diethyl phthalate	84-66-2	<i>Hazard and exposure was verified to be not relevant (CoRAP, 2014)</i>	
A	DiNP	Diisononyl phthalate	28553-12-0 / 68515-48-0	<i>no classification for DINP for either effects on sexual function and fertility, or for developmental toxicity is warranted (RAC, 2018)</i>	REACH Annex XVII, Entry 52
B	DnOP	Di-n-octyl phthalate	117-84-0	Entry on Annex III Inventory: Susp.carcinogen; Susp. hazardous to aquatic environment; Susp. skin sensitizer; Susp. bioaccumulative; Susp. toxic for reproduction	REACH Annex XVII, Entry 52
B	DiDP	Diisodecyl phthalate	26761-40-0 / 68515-49-1	Entry on Annex III Inventory: Susp.carcinogen; Susp. hazardous to aquatic environment; Susp. persistent in the environment; Susp. skin sensitizer	REACH Annex XVII, Entry 52
B	DMP	Dimethyl phthalate	131-11-3	<i>Self classification: Acute tox 3; Eye Irrit.2; STOT SE 3; Skin Irrit. 2; Repr. 2; Aquatic Acute 3; Aquatic Chronic 1</i>	

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Cat.	Abbrev./ Acronym	Systematic name	CAS No.	Classification (CHL/Annex III entry)	REACH Regulation
B	DnPeP	Di-n-pentyl phthalate	131-18-0	CLH: Repr. 1B Aquatic Acute 1 Entry on Annex III Inventory: Susp.carcinogen; Susp. hazardous to aquatic environment; Susp. skin sensitizer; Susp. bioaccumulative; Susp. toxic for reproduction	REACH Annex XIV
B	DCHP	Dicyclohexyl phthalate	84-61-7	CLH: Repr. 1B, Skin Sens.1	CoRAP list; currently under Substance Evaluation for potential ED properties in non-mammal species by SE
B	DPHP	Di(2-propylheptyl) phthalate	53306-54-0		CoRAP list; currently under Substance Evaluation for potential ED properties by DE
B	Hexamoll® DINCH®	Diisononyl cyclohexane-1,2-dicarboxylate	166412-78-8	Entry on Annex III Inventory: Susp.carcinogen; Susp. hazardous to aquatic environment	
C	DiPeP	Di-isopentyl phthalate	605-50-5	CLH: Repr. 1B; Aquatic Acute1	REACH Annex XIV
C	DHNUP	Di-C7-11-(linear and branched)-alkyl phthalate	68515-42-4	CLH: Repr. 1B; Entry on Annex III Inventory: Susp.carcinogen; Susp. hazardous to aquatic environment; Susp. skin sensitizer; Susp. toxic for reproduction	REACH Annex XIV
C	DnHP	Di-n-hexyl phthalate	84-75-3	CLH: Repr. 1B Entry on Annex III Inventory: Susp. carcinogen; Susp.hazardous to the aquatic environment; Susp. mutagen; Susp.persistent in the environment; Susp. respiratory sensitizer; Susp. skin irritant; Susp. skin sensitizer	SVHC on the candidate list, recommended for inclusion on REACH Annex XIV
C	DMEP	Di(methoxyethyl) phthalate	117-82-8	CLH: Repr. 1B Entry on Annex III Inventory: Susp. carcinogen; Susp. hazardous the the aquatic environment; Susp. mutagen	REACH Annex XIV

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21.2.1 Categorisation

according to the Prioritisation Strategy and criteria elaborated under WP4, Year 1 (Deliverable D4.3)

Category A is defined as “substances for which HBM data are sufficient to provide an overall picture of exposure levels across Europe, and interpretation of biomonitoring results in terms of health risks is possible. Risk management measures have been implemented at national or European level. Improvement of knowledge for these substances will therefore focus on policy-related research questions and evaluation of the effectiveness of existing regulatory measures.”

Category B is defined as “substances for which HBM data exists, but not sufficiently to have a clear picture across Europe. Also, knowledge on the extend of exposure, levels and impact on the human health should be improved, in order to give policy makers relevant and strategic data to establish appropriate regulations and improve chemical risk management. Analytical method and capacities to monitor the substances across Europe might have to be improved.”

Category C is defined as “substances for which HBM data scarcely or doesn’t exists. Efforts to develop an analytical method to obtain relevant HBM results need to be done Hazardous properties of the substances are identified, yet greater knowledge on toxicological characteristics and effects on the human health is needed. Interpretation of HBM data is not possible, due to the lack of HBM guidance values.”

Category D is defined as “substances for which a toxicological concern exists but HBM data are not available. HBM4EU research may be focused on the development of suspect screening approaches permitting to generate a first level of data enabling to document the reality of human exposure and better justify further investment in a full quantitative and validated method development.”

Category E is defined as “substances not yet identified as of toxicological concern and for which no HBM data are available. A bottom-up strategy will be applied, consisting to non-targeted screening approaches coupled to identification of unknowns capabilities for revealing, and further identifying, new (i.e. not yet known) markers of exposure related to chemicals of concern for HBM (parent compound or metabolite).”

Justification of Grouping

DEHP, BBzP, DnBP, DiBP, DEP and DiNP were categorised as Category A substances since HBM data is available for at least one country from one of the four geographical regions of the European Union defined by the UN. Furthermore, for all substances well-established analytical methods exist and all substances, except DEP are regulated. DEP and DiNP are the only ones in this category which are not classified as toxic for reproduction 1B. However, Denmark suggested to classify DiNP as toxic for reproduction 1B, which has been rejected by RAC in 2018 (RAC 2018).

DiDP, DnOP, DMP, DnPeP, DCHP, DPHP, Hexamoll®DINCH® were categorised as Category B substances since HBM data is available for some countries, but lacking for at least two of the four geographical regions of the European Union defined by the UN. For most of the substances established analytical methods are available, but might need to be quality assured and/or harmonised. Currently, the metabolites for DPHP and DiDP cannot be distinguished and improvement of analytical methods are needed. Three of these phthalates are regulated and two are currently under evaluation for ED properties.

DiPeP, DHNUP, DMEP and DnHP were categorised as Category C substances, since only little HBM data exists. Methods only exists to measure DHNUP and further research on metabolism and

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feasibility of method development are needed. However, all substances are classified as toxic to reproduction 1B and three of four are on the authorisation list, with DnHP being recommended for inclusion to this list.

21.2.2 Additional information:

DnOP (category B): doesn't exist on the EU market. See page 14 of the ECHA review report: <https://echa.europa.eu/documents/10162/31b4067e-de40-4044-93e8-9c9ff1960715>. However, DnOP metabolites are detected in humans even though to a lesser extent than other phthalates (Frederiksen et al., 2014; Zeman et al., 2013). DnOP do seem to not have antiandrogenic effects, but indications for systemic toxic effects on liver, thyroid, immune system and kidney exist. Due to the scarce data basis, monitoring of the exposure and further research on toxicity is warranted.

DHNUP (category C): It is not registered under REACH. There is almost no information available about this phthalate which suggests that it is not on the market or only has a very marginal market. E.g. Health Canada 2015: stopped at screening assessment because lack of exposure. (<https://www.canada.ca/en/health-canada/services/chemical-substances/challenge/batch-6/dhnup.html>).

DMEP (category C): It is not REACH registered and it is unlikely that there is significant use in the EU.

However, import of goods is not covered by the authorisation of substances under REACH and no restrictions are in effect for these substances. In addition, it has been demonstrated that exposure patterns differ between countries within the EU (Den Hond et al., 2015) and between EU and U.S. (Koch et al., 2017). Therefore, it is suggested to include these substances in the prioritisation list of phthalates to be able to monitor their possible occurrence in human matrices within the European population.

21.3 Objectives / Policy related questions

Exposure characteristics

1. Which are the most sensitive, reliable and cost-effective methods and biomarkers to measure phthalates and Hexamoll® DINCH®?
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?
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®?
5. What are the high exposure groups? (Is there a statistically significant and toxicological relevant difference in mean concentration between adults and children? [...] between occupational exposed and non-exposed adults? [...] between male and female?)

Monitoring the success of existing policy actions and assessing the needs for further regulation

6. Are there different time trends for **less regulated** (DEP, DMP, DCHP, DPHP) and **regulated phthalates** (DEHP, BBzP, DnBP, DiBP, DinP, DnOP) and Hexamoll® DINCH®? (Starting with Cat. A substances for which methods can be standardised in AWP 2)
7. How effective have the different mitigation steps and regulations been for phthalates?

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7a) Had the restriction under REACH the favourable impact, that is a reduction of GM/median concentrations of the already restricted phthalates (DEHP, BBzP, DnBP, DiNP, DiDP, DnOP), especially for children from 2007 until today (2018-2021)?

7b) Was the introduction of the Authorisation obligation under REACH effective enough to protect European citizen? Is there a sufficient decrease of the Cat. A substance levels subject to authorisation (GM/median) in the European population (general/children?) from year 2015 until today (2018-2021) (i.e. DEHP, DnBP, DiBP, BBzP)? Are there differences between countries?

7c) Had the identification as SVHC already an impact on the reduction of the phthalate exposure of the population (i.e. Did the exposure of a certain substance decline after the substance is identified as SVHC)?

Impact on human health

8. Is the exposure to phthalates and their substitutes of health-relevance for the general population and vulnerable groups (inter alia children and pregnant women)? What part of the population has exposure levels exceeding the HBM guidance values - if existing- or TDI)?
9. Does the health relevance depend on age and gender?
10. Can EU wide accepted HBM guidance values be derived for single substances?
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?

Usage of HBM4EU results for policy making

12. How can HBM4EU results feed into the regulatory decisions of ECHA and EFSA?

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Table 21-2: Listing of research activities to be carried out to answer the policy questions concerning phthalates & Hexamoll® DINCH®

Policy Question	Substances	Available knowledge related to policy question	Knowledge gaps / Activities needed to answer policy question
1. Which are the most sensitive, reliable and cost effective methods and biomarkers to measure phthalates and Hexamoll® DINCH®?	Cat. A-C	Methods available to measure the metabolites of all parental compounds, except: DiPeP, DMEP and DHNUP; method exists to measure 21 metabolites covering 11 parental compounds simultaneously but not quality assured- costs differ between countries/labs; not possible to distinguish DiDP and DPHP metabolites	<ul style="list-style-type: none"> - Need to be investigated, for which of the substance a method is available and for which not (WP9, Y1); if quality assured or not & if there is a need for new method development - if there is a need for generating biomonitoring data, cost-benefit-analysis has to be done for determination of phthalates to be measured in national studies (WP8)
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®?	Cat. A	Cat. A substances are expected to be detected in most countries based on experience of DEMOCOPHES; In Year 1 an extensive review will be conducted by Task 7.1 to get an overview of recent studies in the European Union	<ul style="list-style-type: none"> - The Statistical Analysis Group will determine if the existing exposure data are sufficient to derive valid and general statements for the exposure of the European Population and derive reference values, where possible (WP10, Y2) - Studies on metabolisation are needed, in order to develop a method for the following phthalates: DnHP (analogies to DnPeP likely), DHNUP (analogies to HMW (high molecular weight) phthalates such as DEHP DiNP and DiDP likely), DiPeP and DMEP (analogies to DnPeP likely) (WP9) - Prior to method development, it should be determined, if there is a need for the monitoring of Cat. C phthalates in human matrices (Extrapolation of exposure data from other countries, e.g. US, Canada, Asia: WP5, Y2 or prioritisation exercise (WP4, Y2) - Data will be generated in targeted studies and from biobanked samples if available for Cat. B and Cat. C substances (Y3, Y4 in WP8 with preparatory work conducted in WP7 (Y2-Y3)
	Cat. B	Cat. B substances are likely to be increasingly detected in the European Population such as DINP, DPHP & Hexamoll® DINCH®? due to its usage as substitutes for known endocrine disrupter phthalates	
	Cat. C	For Cat. C substances no data on exposure is available and for most no uses are registered within the EU, however exposure patterns differ between countries and authorisation under REACH does not cover import	
3. Do the exposure levels differ significantly between the countries?	Cat. A	Based on the DEMOCOPHES data (sampling year 2011-2012) differences in countries are expected (higher concentration of Cat. A substances in Eastern Europe), but also similar exposure patterns where observed for Germany, Netherlands, Denmark, Israel	<ul style="list-style-type: none"> - The Statistical Analysis Group will determine if the existing exposure data are suitable for comparison between countries (WP10) and if so compare data sets and/ or reference values per country (Y2)

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Policy Question	Substances	Available knowledge related to policy question	Knowledge gaps / Activities needed to answer policy question
	Cat. B	Human Biomonitoring data is scarce (only available for some countries with different population groups measured, e.g. Germany, Sweden)	- We need to answer Question 2 first to be able to recognise differences in exposure depending on geographical origin (Y2-Y3); if not possible to obtain results based on existing data (Statistical Analysis Group, WP10) targeted studies need to be conducted (WP8; Y3-Y5) or targeted analysis in existing biobanked samples should be conducted (WP9; Y2-3)
	Cat. C	For Cat. C substances no data on exposure is available and for most no uses are registered within the EU	- Should be determined, whether there is a need for the monitoring of Cat. C phthalates in human urine (Comparison of exposure data from other countries, e.g. US, Canada, Asia) (WP5; Y2)
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®?	Cat. A & B	Differences of exposure sources exist for HMW phthalates and LMW phthalates: for the first mainly food and indoor air & for the latter others than food; also specific exposure sources exist for DEHP, DiBP (medical devices, medications)	- Exposure determinants for LMW phthalates will be investigated by the Statistical Analysis Group (WP10) to identify sources of exposure per single substance and substance group (Y2) - By means of reverse PBTK modelling identification of the contribution of different routes of exposure to the total exposure can be estimated (WP12; Y2-3)
5. What are the high exposure groups? (Is there a statistical significant and toxicological relevant difference in mean concentration between adults and children? [...] between occupational exposed and non-exposed adults? [...] between male and female?)	Cat. A & B	Based on biomonitoring studies in Europe (DEMOCOPHES project and others) it can be observed that children are exposed to a higher extend than adults; only slight differences expected for male and female population; data on the exposure of workers is lacking, but a higher exposure to phthalates are assumed	- Need to be investigated whether there is a difference in the exposure levels of male and female population by comparing exposure levels based on existing data (Statistical Analysis Group, WP10; Y2) - Statistical Analysis Group will also determine different exposure levels of different age groups depending on the data available (WP10; Y2) - Need to be determined whether data on occupational population is existing (WP7; Task 7.1; Y1); if so Statistical Analysis Group will also determine different exposure levels of occupational population in comparison with general population (WP10; Y2) - Targeted occupational studies for phthalate exposure (e.g. in plastic and construction sectors) will be planned (WP8; Y2) - Occupational exposure will be estimated (WP12, Y4)

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Policy Question	Substances	Available knowledge related to policy question	Knowledge gaps / Activities needed to answer policy question
<p>6. Are there different time trends for unregulated (DEP, DMP, DCHP, DPHP) and regulated phthalates (DEHP, BBzP, DnBP, DiBP, DinP, DnOP) and Hexamoll® DINCH®?</p> <p>7. How effective have the different mitigation steps and regulations been for phthalates?</p>	Cat. A & B	Time trend analysis in Germany, Sweden, Flanders suggest a decline of regulated phthalates (e.g. DEHP, DiBP; BBzP, DnBP), whereas DiNP & DPHP & the substitute Hexamoll® DINCH®? show an increasing trend	<ul style="list-style-type: none"> - Does this apply to other countries as well? Are time trends available for other countries? To answer these questions gap analysis need to be performed (WP7; Y1) and comparability of data available need to be evaluated and analysed (WP10; Y2) - Do the different regulation steps (Identification as SVHC; Restriction, Authorisation) have a direct impact on the phthalates exposure patterns in the EU population? (WP10) - Does the authorisation process stimulates substitution? (WP10) - Is the current regulation system effective enough to reduce exposure to phthalates? How affect the different regulation steps the exposure patterns? Are there differences? Do we need further regulation to reduce the health-risk? (WP10, WP5)
<p>8. Is the exposure to phthalates and their substitutes of health-relevance for the general population and vulnerable groups (inter alia children and pregnant women)? What part of the population has exposure levels exceeding the HBM guidance values - if existing- or TDI)?</p>	Cat. A & B	German HBM values are available for the sum of DEHP metabolites, Hexamoll® DINCH®? metabolites, DPHP metabolites; BE values exist for sum of DEHP metabolites	<ul style="list-style-type: none"> - Compare current exposure data (reference values) with EU health-based guidance values, if available (derived in WP5→ Hexamoll® DINCH®, DEHP: Y1; DPHP, DiBP, BBzP, DnBP: Y2;) or if not available other as German HBM values and BE values - Exposure and specific determinants will be assessed and compared to toxicological threshold values and vulnerable groups and geographical hot spots where policy actions are required can be identified using PBTK modelling (WP12, Y2-3)

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Policy Question	Substances	Available knowledge related to policy question	Knowledge gaps / Activities needed to answer policy question
9. Does the health relevance depend on age and gender?	Cat. A	Sensitive groups for health effects resulting from phthalates exposure are pregnant women and their foetuses based on the endocrine disrupting effects on the male reproductive tract development and effects on neurodevelopment (both male and female infants and young children); phthalates exposure is also associated with obesity in older women	<ul style="list-style-type: none"> - Epidemiological studies investigating effects on neurodevelopment in infants and children/male reproductive tract development should be compared and statistically analysed (meta-analysis) to strengthen the correlation of age, gender and other determinants with single/cumulative substance exposure (WP11, WP10) - Use of AOPs to establish associations of health outcomes and exposure (WP13; Y3-4) - Estimation of the burden of disease of phthalates and EDCs in general for EU population (WP5)
	Cat. B/C		<ul style="list-style-type: none"> - Same can be assumed for Cat.B&C substances, if reprotoxic potential is given; for Hexamol® DINCH® and other phthalates thought to be no endocrine disrupting chemicals health-relevant determinants should be investigated (WP10, WP11, WP13)
10. Can EU wide accepted HBM guidance values be derived for single substances and for the additively acting phthalates?	Cat. A & B	For some single substances German HBM values exist and within 1 year, it can be determined whether EU wide health-based guidance values can be derived	<ul style="list-style-type: none"> - WP5 will develop a concept to derive EU wide health-based guidance values (Y1) and will determine for which single substances it is possible and develop a time frame for the derivation of EU wide health-based guidance values (Y1) - In Y2-Y3 WP5 will examine the possibility of an aggregated (EU-wide) health-based guidance value for phthalates in close collaboration with WP15 - WP15 will further develop on the scientific basis for a cumulative risk assessment
	Cat. C	For those substances, exposure data is scarce, if available at all. No BE or German HBM values exist.	<ul style="list-style-type: none"> - Lack of exposure data makes it difficult to derive health-based guidance values → need to be determined, whether it is suitable and useful to derive values for Cat. C substances (WP5; Y2-Y5) based on gap analysis (WP7, task 7.1; Y1)
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?	Cat. A-C	Since many relevant phthalates have similar toxicological profiles, a cumulative assessment is important in order to not underestimate risks. A decision is needed on which substances of the phthalate group should be included in such an additive approach. Basis for this should be similar adverse effects (function that is disrupted) and not only the mode of action.	<ul style="list-style-type: none"> - Basis for a cumulative risk assessment should be similar adverse effects (function that is disrupted, here anti-androgenic effects) and not only the mode of action. (WP15) - WP5 will in close collaboration with WP15 address the risk emerging for combined phthalate exposure (Y4) and explore health risk assessment (HRA) of combined exposure for phthalates

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Policy Question	Substances	Available knowledge related to policy question	Knowledge gaps / Activities needed to answer policy question
12. How can HBM4EU results feed into the regulatory decisions of ECHA and EFSA?	Cat A & B		<ul style="list-style-type: none"> - EU-wide health-based guidance values will be derived for phthalates (WP5, Y1-2), which are a useful tool to determine if a concern to human health exist for the exposure to phthalates and therefore measures need to be taken - In WP5 the improved use of HBM in health risk assessment (HRA) and in health impact assessment (HIA) for phthalates will be explored (Y1) and case studies will be performed on the integration of HBM information in HRA and HIA for phthalate substitutes as DPHP and Hexamoll® DINCH® including the EU-wide consolidated HBM guidance values and reference values (WP10) in Y2 - Method for HBM-based indicators will be developed (WP5; Y1) and applied on single substances and the use of substance groups (as phthalates) will be addressed (WP5; Y2) - Instruments to link health and exposure (WP13; WP14) and to better estimate risks from exposure (WP12) will be explored and their suitability in risk assessment and management will be evaluated (e.g. cumulative risk assessment; WP15)
13. What is the economic impact of phthalates and substitute exposure?	Cat A	Legler et al., 2015 estimated obesity, diabetes and associated costs attributed to EDCs including phthalates in the EU. According to their calculations exposures to phthalates contribute substantially to obesity in older women, with a moderate probability of €15 billion costs per year	<ul style="list-style-type: none"> - In WP5 health impact assessment (HIA) for phthalates (single substances and cumulative) will be explored: it should be discussed whether the cost estimation should be included together with the burden of disease estimates and how this can feed into the HIA

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21.3.1 Additional Comments:

- a) Surveyed data to be analysed or published (in English)
- ▶ Spain: Adult working population, 18-65 years, n = 1880. Nation-wide representativeness. March 2009-July 2010
 - ▶ Sweden: DEHP, DnBP, BBzP and DEP: Time series on children and population based study from 2010-2011 (Bjermo et al., report in Swedish)
 - ▶ Belgium: FLEHS3: Data surveyed for DiBP and DEP
- b) Data (representative for population) expectable from:
- ▶ France: Esteban (running since 2nd half of 2016): DEHP, DnBP, BBzP, DEP, DiNP, DnOP, DMP, DCHP
 - ▶ Finland: FinHealth from 2017 on. 6000 samples planned: all Cat A substances and DiNP, DiDP, DnOP, DCHP, DPHP
 - ▶ Germany: GerES V (children): 2015-2017, all Cat A and B substances
- c) Information for Pillar 3:
- ▶ Like it was mentioned in the Background Information some phthalates (3 to 8 carbon atoms in the backbone of the side chain) have or are suspected to have anti-androgenic properties and as such induce developmental and reproductive malfunctions in rodent studies (phthalate syndrome). Those disturbances include malformations of the epididymis, vas deferens, seminal vesicles, prostate, external genitalia (hypospadias), and cryptorchidism (undescended testes) as well as retention of nipples/areolae (sexually dimorphic structures in rodents) and demasculinisation of the perineum, resulting in reduced anogenital distance (AGD). Those effects can be ascribed to a disturbance of fetal testicular Leydig function, which results in significant reduction of testosterone levels. (Gennings et al., 2014) Also the production of insulin like factor 3 in Leydig cells is disturbed by phthalates, which also causes anti-androgenic effects.

Those effects are similar to the ones subsumed under the human testicular dysgenesis syndrome. However, there are no resilient data on humans, which is why epidemiological studies should try to prove the associations between a phthalate burden and adverse health effects. Data from birth cohorts would probably meet some of the requirements, since the discussion on whether the health impacts can be traced back to in utero exposure or to exposure during childhood could be furthered. Among other endocrine effects in adults (differing for male and female individuals), phthalates are furthermore associated with respiratory problems and effects on blood pressure. For Category C phthalates a first step should be an assessment concerning the relevancy (are the people in Europe exposed or not?), before starting research activities in pillar 3 on those substances.

21.4 References

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22 Prioritised substance group: UV filters

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22.1 Background information

22.1.1 Hazardous properties

Benzophenone 3 (BP-3) displays a low acute toxicity profile. It is not considered as being irritating to the skin and the eyes¹. Results from animal studies—primarily dietary studies that affected body weight gain—showed alterations in liver, kidney, and reproductive organs in rats and mice with BP-3 administered dermally and orally².

BP-3 is on the Community Rolling Action Plan (CoRAP) list because of potential endocrine disruption³. BP-3 elicited anti-androgenic activity in a human breast carcinoma cell line⁴ and interferes with functions of human sperm cells in vitro. Critical effects are maternal and developmental toxicity⁵. In cell cultures, BP-3 (and also BP-8) were found to affect lipid metabolism⁶. Larval zebrafish exposed to environmental concentrations of BP-3 showed developmental neurotoxicity, altered motor and social behaviors, in addition to changes in cell proliferation and apoptosis in the larval head region⁷. In female mice, low dose exposure causes long-lasting alterations to mammary gland morphology and function⁸. Dermal exposure of pregnant mice to low doses of BP-3 (during early pregnancy) resulted in intrauterine growth restriction phenotype and in disturbed sex ratio (more female offspring)⁹. Prenatal exposure of pregnant mice to BP-3 impairs autophagy, disrupts several signalling pathways and also alters epigenetic and post-translational statuses in brain neurons¹⁰. Studies in rat primary cortical neuronal cultures and neuroblastoma cell lines showed decreased cell viability after BP-3 treatment at moderate concentrations¹¹. In addition, exposure at birth of rats to BP-3 perturbs early events of germ cell development, and alters early follicular assembly¹².

In a study on young men from Spain, there was a significant positive association between urinary BP-3 concentrations and serum FSH levels¹³. In male adolescents in the US, urinary BP-3 was associated with lower total testosterone¹⁴. In a study of young Danish men, associations between male reproductive health parameters and urinary levels of benzophenones such as BP-3, BP-1 and 4-HBP were observed in filaggrin gene mutation carriers but not in controls¹⁵. In a study in healthy, premenopausal women, UV filter factors (BP-1, BP-3) were associated with decreased estradiol, FSH, and LH¹⁶. In pregnant women from the Boston area, elevated urinary concentrations of phenols including benzophenone-3 were associated with increases in the urinary oxidative stress biomarkers 8-OHdG and 8-isoprostane¹⁷.

Exposure to BP-3 was not associated with preterm birth¹⁸, but was associated with decreased birth weight and length (in girls only)¹⁹.

Benzophenone is possibly carcinogenic to humans (Group 2B, IARC classification, based on sufficient evidence in experimental animals)⁴ Benzophenone exerts tumourigenic effects in rats and mice in the liver, the kidney and in the haematopoietic system, including rare histiocytic sarcomas. Available evidence supports that benzophenone is not genotoxic. Benzophenone meets the criteria for classification as carcinogenic in category 2²⁰. Benzophenone may alter endocrine signalling through multiple effects on receptors. Critical effects are liver and kidney effects.

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Benzophenone-1 (BP-1) is a UV filter and metabolite of BP-3. BP-1 is not irritating nor sensitizing at concentrations that may be found in cosmetic products. The toxicity studies available indicate low acute and subchronic toxicity of BP-1. BP-1 is not mutagenic. The lowest effect levels were determined for reproductive toxicity with lowest observable adverse effect levels (LOAELs) between 100-625 mg/kg and NOAELs between 100-250 mg/kg. BP-1 is on the European Commission priority list of potential endocrine disruptors²¹.

In a study of young Danish men, associations between male reproductive health parameters and urinary levels of benzophenones such as BP-3, BP-1 and 4-HBP were observed in filaggrin gene mutation carriers but not in controls. In a study in healthy, premenopausal women, UV filter factors (BP-1, BP-3) were associated with decreased estradiol, FSH, and LH¹⁵⁻¹⁶

In the a Spanish sub-cohort from the European Prospective Investigation into Cancer and Nutrition study, a negative trend between BP1 and type 2 diabetes mellitus risk was observed in women²².

Benzophenone-2 (BP-2) is a UV filter commonly used in personal care products. BP-2 may disturb thyroid hormone homeostasis by inhibiting or inactivating thyroid peroxidase, effects that are even more pronounced in the absence of iodide²³. Both BP-2 and BP-3 were shown to exert uterotrophic effects and BP2 was shown to bind to estrogen receptors²⁴. In fish and mammals, BP-2 induces a variety of reproductive disorders, including feminization of male fish, inhibition of gamete development in fish, reduction of testosterone secretions from testicular tissue, induction of uterotrophic effects in rats, changes in bone density and osteo-regulation, changes in luteinizing hormone, cholesterol levels, fat deposition, and an increased risk of endometriosis²⁵. BP-2 was also found in the brains of rats that were dermally exposed to BP-2, this shows that BP-2 passes through the blood-brain barrier²⁶. In a study on exposure to UV filters and fertility, male partners' concentrations BP-2 was associated with reduced fecundity²⁷.

4-Methylbenzylidene camphor (4-MBC) is found in cosmetics and in drinking water. The available data suggest no genotoxicity, mutagenic potential or phototoxicity of 4-MBC. However, this chemical is suspected to have a mild endocrine disrupting effect on the thyroid gland. Experiments in rats found 4-MBC to have development toxicity^{21,28}. A recent study that exposed human cell culture (trophoblast cells) to 4-MBC showed that this chemical has the potential to delay the normal growth and survival of tissue and may hamper normal placental formation during early pregnancy²⁹. Exposure of zebrafish embryos to 4-MBC induced morphological abnormalities during embryonic development, including notochord curvature, delayed absorption of yolk sac and pericardial oedema, in addition to the decrease in embryo heart rate³⁰.

3-benzylidene camphor (3-BC) - 3-BC is a potential endocrine disrupter³¹. Experiments in vivo and in vitro revealed oestrogenic activity. In addition, 3-BC was found to interrupt sexual development and maturation in animal models. According to the Scientific Committee on Consumer Safety, hormonal activities of 3-BC have been reported in vitro: estrogenic and anti-estrogenic effects as well anti- androgenic activities. In vivo, the expression of target genes (ER α , ER β , SRC-1 and PR (progesterone receptor)) has been shown to be altered in both males and females rats³².

4-hydroxy benzophenone (4-HBP) is used as an industrial UV-filter. 4-HBP has potential to disrupt endocrine activity, and fetal growth. 4-HBP exposure in women carrying a male fetus was associated with increased maternal thyroid hormone concentrations, in addition to decreased birth outcomes (lower weight and shorter head and abdominal circumferences at birth compared to the low exposure group)³³.

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4-methylbenzophenone (4-MBP) is used in paints and varnishes, in food packaging but not in cosmetics. According to an assessment by EFSA, the currently available data on 4-methylbenzophenone are insufficient to enable the assessment of this substance with respect to its human toxicological effects. 4-MBP is expected to be a non-genotoxic carcinogen³⁴.

Hazardous Properties of Benzophenones

	Critical effect	Potential Endocrine Disruption	Other
BP-3	Maternal and reproductive toxicity	Suspected	Developmental neurotoxicity
BP	Liver and kidney	Suspected	Possible carcinogenic in human (IARC)
BP-1		Suspected	
BP-2		Suspected	
4-MBC	Repeated dose: thyroid effects	Suspected	
3-BC		Suspected	
4-HBP		Suspected	
4-MBP			Expected carcinogen (EFSA)

22.1.2 Exposure characteristics

Benzophenone is manufactured and/or imported in the European Economic Area in 1000-10000 tonnes per year; it is used by consumers, by professional workers (widespread uses), in formulations or re-packaging and at industrial sites.

Benzophenones are used in cosmetics and in personal care products, food contact materials, coating products, fillers, modelling clay and finger paints. UV-absorbers and UV filters including benzophenone-1 and benzophenone-3 are added to food packaging to protect the packaging itself and the contained food from harmful UV light⁵.

Release to the environment is likely to occur from: industrial use, indoor use (e.g. machine wash detergents, personal care products, paints and coating, fragrances and air fresheners).

Biological half-life (urine) of 16 hours

Human Biomonitoring (HBM) data: pregnant women in US (California)³⁵, France³⁶, China³⁷, Israel³⁸, general public in Belgium³⁹, Denmark⁴⁰, and the US⁴⁶. Data on exposure in children is available for the US⁴¹, Denmark⁴², China⁴³, Australia⁴⁴, and Taiwan⁴⁵. Overall, BP-3/BP-1 exposure data is much more limited for children and adolescents compared to adults.

Several biomonitoring studies (including NHANES) have focused on BP-346. BP-3 has been widely detected in several biomonitoring studies with urinary levels correlated with the use of personal care products. Higher BP3 exposure has been observed in the female population, possible due to its presence in personal care products⁴⁶.

In women undergoing fertility treatments, self-reported sunscreen use, physical activity, and time spent on moderate/heavy outdoor work were positively associated with urinary benzophenone-3⁴⁷. In pregnant Chinese women, urinary levels of benzophenones were associated with the

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refurbishment of homes and household income, and higher levels of benzophenones were observed in summer than in winter⁴⁸. In women in Michigan (U.S), sunscreen use was strongly positively associated with benzophenone-3 concentrations. Benzophenone-3 concentrations tended to be highest in the summer (25.4%) and lowest in the autumn (-20.5%) compared with winter⁴⁹.

22.1.3 Policy relevance

Since September 2017 the use of BP-3 in the EU is restricted to 6% in cosmetic sunscreen products and up to 0.5 % in other cosmetic products⁵⁰. In February 2020, the European Commission requested the SCCS to carry out a safety assessment on Benzophenone-3 in view of new information provided in a "Call for data on ingredients with potential endocrine-disrupting properties used in cosmetic products".

According to the Cosmetics Regulation (EU Regulation 1223/2009), BP-4 and BP-5 are permitted as UV filters in cosmetic products. 4-MBC is allowed as a UV filter in cosmetic products with a maximum concentration of 4% in ready-for-use preparations⁵¹.

According to the Scientific Committee on Consumer Safety, the use of 3-BC as a UV-filter in cosmetic products in a concentration up to 2.0% is not safe³².

Benzophenone is approved as an additive in plastic food contact materials, with a specific migration limit of 0.6 mg/kg⁵². In September 2019, the USA amended food additive regulations to no longer authorise the use of benzophenone as synthetic flavoring substances for use in food and to no longer provide for the use of benzophenone as a plasticizer in rubber articles intended for repeated use in contact with food. According to the FDA, this action was taken in response to evidence that the additive causes cancer in laboratory animals, and despite the determination "that these substances do not pose a risk to public health under the conditions of their intended use"⁵³.

Inks are not covered by a specific European legislation on food contact materials. The use of printing inks has to comply with the general rules of Regulation (EC) No 1935/2004 and with good manufacturing practice as laid down in Commission Regulation (EC) No 2023/2006.

22.1.4 Technical aspects

BP-3 can be directly measured and quantified in urine in HBM studies. In addition, three oxidative metabolites (2,4-dihydroxylbenzophenone, 2,2'-dihydroxy-4-methoxybenzophenone, and 2,3,4-trihydroxybenzophenone) can also be measured in HBM studies using quantitative analytical methods⁵⁴.

On-line TurboFlow-LC-MS/MS method has been developed at Copenhagen University Hospital for simultaneous biomonitoring of nine UV filters in urine (benzophenone, benzophenone-1 benzophenone-2, benzophenone-3, 5-chloro-2-hydroxybenzophenone, 4-hydroxybenzophenone, 4-methyl-benzophenone, 3-(4-methylbenzylidene)-camphor, and 3-benzylidene camphor)⁵⁵.

22.1.5 Societal Concern

UV filters, including benzophenones, are widely used in cosmetics, personal care products, food contact materials, inks, textiles and other consumer products. Therefore, there is a high potential for the general public (including vulnerable populations) to be exposed to benzophenones.

While UV filters in sunscreens and cosmetics have been effective in protecting against a variety of UV-related pathologies, such as sunburns and melanomas, growing popularity of sunscreens and increasing potential exposure has led to increased societal concern about their potential impact on the environment and human health.

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There are several EU regulations regarding benzophenones, such as the restriction of BP-3 to 6% in cosmetic sunscreen products and to 0.5% in other cosmetic products. However, there are regulatory gaps regarding benzophenones. There are also knowledge gaps regarding the exposure pathways and health effects in humans of many of the benzophenones. BP-3 was included in the Community Rolling Action Plan list because of potential endocrine disruption and fulfilling exposure criteria³.

BP, BP-2 and BP-3 are on the SIN (“Substitute It Now”) list.

In addition, CHEMTrust nominated this group of chemicals as a priority substance for HBM4EU. In 2018, the Environment Working Group (EWG) reviewed studies and documents regarding UV filters and recommended a thorough investigation of the safety of all ingredients currently in sunscreens to ensure that none of them damage skin or cause other toxic effects in consumers. Because of concerns regarding potential health effects, the EWG has recommended that consumers avoid sunscreens with oxybenzone. It is noteworthy that consumer avoidance of sunscreens because could increase public health risk from UV rays (sunburn and skin cancers); **therefore risk- benefit analysis and risk communication is especially important with regards to benzophenones.**

Of note, due to reports on adverse effects of UV filters on coral reef, there is societal concern about ecological effects of sunscreens.

22.2 Categorisation of Substances

Table 22-1: Substances included in the substance group, listed according to availability of toxicology and human biomarker data, in category A, B, C, D, E substances (see general introduction)

Category	Abbreviation/ Acronym	Systematic name	CAS No.	Regulation
B	BP-3	Benzophenone-3	131-57-7	Cosmetics 2017/238
C	BP	Benzophenone	119-61-9	Plastic materials in contact with food 2002/72
C	BP-1	Benzophenone-1	131-56-6	
C	BP-2	Benzophenone-2	131-55-5	
C	4-MBC	3-(4-methylbenzylidene)-camphor	36861-47-9	
C	3-BC	3-benzylidene camphor	15087-24-8	
C	4-HBP	4-hydroxy-benzophenone	1137-42-4	
C	4-MBP	4-methyl-benzophenone	134-84-9	

Justification of Grouping

We propose to categorise BP-3 in Category B, as European HBM data are available from some countries. Understanding of sources of human exposure is limited. For BP-3, there is a need for improved understanding of exposure levels and potential health impacts to inform policy makers.

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For the remaining substances, we propose to categorise them as Category C as HBM data is scarce. While analytical methods have been developed, there is a need for validation and widespread collection of data using validated methods.

22.3 Policy-related questions

1. Are sensitive, reliable and cost effective methods and biomarkers available to measure UV filters?
2. What are current exposure levels to benzophenones in the EU population (cumulative exposure from different exposures sources)?
3. What are the major sources of exposure to benzophenones in the EU population and in vulnerable groups such as children and pregnant women? (cosmetics and personal care products, plastic and other food contact materials, other)
4. Do exposure levels differ significantly between different EU countries (possibly related to climate)?
5. Do exposure levels differ between different sub-groups: elderly, adults, and children? between males and females? Between adults of different age groups? Between individuals in different ethnic subgroups (perhaps due to differences in use of sunscreen products)?
6. Are current exposure levels safe in relation to the endocrine and carcinogenic properties of benzophenones? (for the general population and for vulnerable groups such as children and pregnant women)

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22.4 Research Activities to be undertaken

Table 22-2: Listing of research activities to be carried out to answer the policy questions

Policy question	Substance	Available knowledge	Knowledge gaps and activities needed
1. Are sensitive, reliable and cost effective methods and biomarkers available to measure UV filters?	Benzophenones	List of biomarkers, matrices, analytical methods, and candidate laboratories is available (WP 9)	WP9 - Update list of biomarkers, matrices, analytical methods and candidate laboratories as needed
2. What are current levels of exposure of the EU population to benzophenone UV-filters?	Benzophenones, emphasis on BP-3	There are ~30 published HBM studies reporting BP-3/BP-1 exposure data in European countries, with the largest number of studies in Denmark. BP-3/BP-1 exposure data is much more limited for children and adolescents compared to adults.	Knowledge gaps: current level of exposure to benzophenones other than BP-3 and BP-1 WP7 – Update of questionnaires on available data and studies as needed WP10 - Identifying additional data collections and uploading metadata and aggregated data to IPCHEM WP12 - Modelling, estimate exposure levels to benzophenones, differences within countries WP8 - Generate new HBM data from aligned studies to fill identified data gaps
3. Do the exposure levels differ significantly between the countries?	Benzophenones, emphasis on BP-3	No. Available data from literature on average urinary BP-3 levels from studies from Western Europe (4 studies), Southern Europe (1 study) and Northern Europe (19 studies) were compared in a random-effects meta-regression model. No significant difference in average urinary BP-3 levels between the three regions were observed when adjusting for sex, age and period of sample collection (WP13)	Knowledge gaps: Only 1 study identified in Southern Europe, no studies with data on urinary BP-3 were identified from Eastern Europe WP13 / WP14 - Publication of a systematic integrative review on BP-3/BP-1 exposure levels WP8 - Generate new HBM data from aligned studies to fill identified data gaps WP10 – Statistical analysis plan for data obtained in the aligned study (geographical comparisons, exposure distributions and calculating European exposure values, if possible)

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Policy question	Substance	Available knowledge	Knowledge gaps and activities needed
4. What are the main sources of exposure to benzophenones?	Benzophenones	Based on review of limited literature, main exposures sources include sunscreen, make-up products and personal care products. The occurrence of BP-3 at relatively high levels has been described in indoor dust, and to a lower extent in textiles, indoor air and tap water. Although benzophenones could be used in plastics and food contact materials to protect them from UV radiation, there is little published data available.	<p>Knowledge gaps: Literature available only on BP-3; little available evidence on food contact materials as source of exposure</p> <p>WP8 – Collect new data on exposure sources as part of aligned studies</p> <p>WP10 – Statistical analysis plan for data obtained in the aligned study (exposure determinants)</p> <p>WP13 – Publish review of available literature on exposure sources</p>
5. Who are the highest exposed groups? Are there statistical differences in concentration between different ages? males and females? Ethnic subgroups? occupational vs. general population exposure.	Benzophenones, emphasis on BP-3	Exposure distributions for BP-1, BP-3 and DHMB in urine, stratified by age and gender is available (WP 10). Reported average BP-3 levels in urine stratified by age and gender based on the literature is available for Northern Europe (WP13). No significant difference was seen between males and females in European studies. Average urinary BP-3 levels were significantly lower in children and adolescence compared to adults.	<p>Knowledge gaps: Age and gender differences in Southern and Eastern Europe countries not available</p> <p>WP8 - Aligned HBM studies on benzophenone exposure</p> <p>WP10 – updated report on exposure distributions</p>
6. Are current exposure levels safe?	BP-3 and BP-1	Appropriate effect biomarkers were identified Review on the available literature on Benzophenone-3 and -1 has been performed including evaluation of toxicology studies, HBM data and exposure-health data, includes effect biomarker data and health outcome data, in vitro and in vivo data, intervention studies, xenometabolism.	<p>Knowledge gaps: The current research is not sufficient to answer this question</p> <p>WP5:Risk assessment of BP-3 based on urinary levels and effect levels</p> <p>WP12: Development of integrated exposure modelling platform, PBTK modelling</p> <p>WP13 – Continue review of cohorts available to link BP-3 exposure-health associations</p> <p>WP13 – WP14 - Comparison of toxic doses and HBM data is ongoing</p>

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Policy question	Substance	Available knowledge	Knowledge gaps and activities needed
7. How can HBM4EU results feed into regulatory decisions and risk assessments (ECHA and EFSA)?	UV filters, specifically BP-3		WP5 – Risk Assessment started with data from available HBM studies
6. How effective was the restriction of BP-3 in reducing exposures in the EU population?	BP-3 and other benzophenones	Since September 2017 the use of BP-3 has in EU been restricted to 6% in cosmetic sunscreen products and up to 0.5 % in other cosmetic products	WP10 – Compare between exposure to regulated UV filters (BP-3) and nonregulated UV filters

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Table 22-3: Summary of biomonitoring studies on UV filters

Study / Institution	Country	Year of publication	Study Population	Matrix	Analytes	Citation + link
NHANES	USA	2003-2004	General, includes children	Urine	BP-3	Calafat et al.
NHANES	USA	2003-2010 (sample collection)	General, includes children	Urine	BP-3	CDC Report
Bispebjerg Hospital	Denmark	2004	General	Urine, plasma	BP-3, 4-MBC	Janjua et al.
Princess Alexandra Hospital	Australia	2005	Human skin culture	Skin	BP-3, Octocrylene	Hayden et al.
Sahlgrenska University Hospital	Sweden	2006	General	Urine	BP-3	Gonzalez et al.
Southe Korean institutes	South Korea	2010-2011	General	Urine	BP-1, BP-2, BP-3, BP-4, BP-8	Kang et al
Maternal and Infant Environmental Exposure Project (MIEEP)	USA	2010-2011 (sample collection)	Pregnant women and infants	Urine	BP-3	Biomonitoring California
Biomonitoring Exposures Study (BEST) – Pilot Study and Expanded Study	USA	2011-2012 (sample collection)	Adults	Urine	BP-3	Biomonitoring California
State University of New York at Albany	USA	2012	Woman	Urine	BP-1, BP-3, , BP-2, BP-8	Kunisue et al
Institut Albert Bonniot	France	2012	Mothers giving birth	Urine	BP-3	Philippat et al
Nankai University	China	2013	children, adults, and pregnant women	Urine, blood	BP-1, BP-2, BP-3, BP-8, 4OH-BP	Zhang et al
Institut Albert Bonniot	France	2013	Pregnant women	Urine	BP-3	Philippat et al
University of Copenhagen	Denmark	2013	Children	Urine	BP, BP-1, BP-2, BP-3, BP-7, 4-MBP, 4-HBP, 4-MBC, 3-BC	Krause et al
Queensland	Australia	2015	Children and adults	Urine	BP-3	Heffernan et al.

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Study / Institution	Country	Year of publication	Study Population	Matrix	Analytes	Citation + link
Several universities	China	2015	Young children	Urine	BP, BP-1, BP-2, BP-3, BP-8, 4-HBP	Gao et al
University of Liege	Belgium	2014	Adults	Urine	BP-3	Dewalque et al.
Several universities	Denmark	2017	General	Urine	BP-1, BP-3	Morrison et al
I-Shou University	Taiwan	2017	Children and adolescents	Urine	BP-3	Chang et al
Copenhagen University Hospital	Denmark	2017	Children and adolescents	Urine	BP, BP-1, BP-2, BP-3, BP-7, 4-HBP, 4-MBP, 4-MBC, 3-BC	Frederiksen et al.
University of Bath	UK	2018	General (samples collected from a festival event)	Urine	BP-1, BP-2, BP-3, 3-BC, Homosalate, Octocrylene	Lopardo et al
Pregnant women in Israel	Israel	2018	Pregnant women	Urine	BP-3	Machtinger et al
EURO-MIX study	Norway	2019	Adult population	24 hr urine, blood	oxybenzone/ benzophenone-3	Husoy et al.
Sample pooling/ misclassification	France	2019	Pregnant women	Urine	BP-3	Vernet et al.
LIFECODES	USA	2019	Pregnant women	Urine	BP-3	Ferguson et al.
Environment and Reproductive Health cohort study	USA	2019	Women undergoing fertility treatment	Urine	BP-3	Minguez-Alarcon et al.
Universidade de São Paulo	Brazil	2019	New detection method	Saliva	Benzophenones (3, 1, 2, 8, 4-OH BP)	De Oliveira et al
College of Public Health and Human Sciences	USA	2019	Children and adults (>=6 years old) – exposure profiles	Urine	BP-3	Przybyla et al
South China Normal University	China	2019	Children (4-6 years old)	Urine	BP1, BP2, BP3, BP4	Li et al
University of Granada (GraMo cohort)	Spain	2019	adults	adipose	BP-3	Artacho-Cordón et al.

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Study / Institution	Country	Year of publication	Study Population	Matrix	Analytes	Citation + link
Chinese Center for Disease Control and Prevention	China	2019	Pregnant women	Urine	4-OH-BP, BP-1, BP-3, TCS	Li et al.
Huazhong University of Science and Technology, Wuhan, Hubei	China	2019	Pregnant women	Urine	4-OH-BP, BP-1, BP-3	Long et al
HERMOSA study	USA	2019	adolescents	Urine	BP-3	Berger et al
Norwegian Institute of Public Health + Grenoble	France, Norway	2020	Pregnancy + first year (coupled)	Urine	BP-3	Rolland et al.
Study of Environment, Lifestyle, and Fibroids	USA	2020	Women, ages 23-34	Urine	BP-3	Betha et al.
Copenhagen University Hospital	Denmark	2020	General, young males	Paired urine, serum, and seminal fluid samples	BP-3	Frederikson et al.
Granada EPIC-Spain cohort	Spain	2020		Serum	BP-1, BP-3	Salamanca-Fernandez et al.
Massachusetts General Hospital (MGH) Fertility Center	US	2020	Fertility clinic	Urine	BP-3	Mustieles et al.
Women FFs Biomonitoring Collaborative	US	2020	Women, firefighters and office workers	Serum	"serum suspect screening" BP-3 confirmed	Grashow et al.
Copenhagen University Hospital	Denmark	2020	Young men, Danish population	Urine	BP-3	Frederikson et al.
George Mason University	US	2020	Women of reproductive age	Urine	BP-3, BP-1	Pollack et al.
Southern China	China	2020	Matched maternal-fetal samples	Urine, serum, amniotic fluid	4-OH-BP, BP-1, BP-3, BP-8	Song et al.
SEPAGES study group	France	2020	8 pregnant women	Urine	BP-3	Nakiwala et al.

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