HBM4EU science and policy for a healthy future

HORIZON2020 Programme
Contract No. 733032 HBM4EU

Scoping documents for the second round priority substances

Deliverable Report

D4.6

WP4 - Prioritisation and input to the Annual Work Plan

Deadline: November 2018

Re-Upload by Coordinator: 11 March 2019

Entity	Name of person responsible	Short name institution	Date [Received]
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2 Categorization 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.

- **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.

3 Abstract/Summary

This deliverable contains scoping documents for the second round substances that were prioritised in HBM4EU. For each substance group, scoping documents are produced under Workpackage 4.4 of HBM4EU. The scoping documents contain a review of the available evidence, list policy-related questions, identifiy knowledge gaps and propose research activities. Proposed activities are fed into the framework of work packages and tasks of HBM4EU in a coordinated and harmonized 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 optimize 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.

4 Introduction

HBM4EU has established a strategy for deriving prioritized 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.

5 Prioritized 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: CH_2=CH-CO-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. 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-catalyzed hydrolysis and it does not bioconcentrate in aquatic organisms (10).

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 stabilizer 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 assumend 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 ($BMDL_{10}$). From animal studies, $BMDL_{10}$ 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. 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-neopastic 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 $1x10^{-4}$ and $1x10^{-6}$, 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/m^3 and short term (acute) 120 mg/m^3; 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 harmonized 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).

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 characterized 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 organizations. Chemsec, an independent organization 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 organizations (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 Categorization 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

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 carcigonogens 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?

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?	Acrylamide	2) German Environmental Survey V, 2014-2017, general population representative date for 3-17 year old children and adolescens 3) German study, general population, n=999, 2003-2004 n=999 (18). 4) NewGeneris: Danimark, Norway, Greece, England, Spain: mother-child 2006-2010 n=1,151 (5). 5) MoBa: Norway mother-child, 1999-2008, n=79 (6). 6) CAPS: Swedish case-control study for cancer, 2001-2002, n=330 (17) 7) Swedish occupational exposed, 1997-1998 n=210 (13, 14) 8) Danish post-menopausal cohort, n=740 (15, 16)	Lack of HBM data of the general population for most of the EU countries. Actions: - 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) **Relevant WPs: WP7 WP8 WP10**
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? 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 needs to be discovered (e.g. smoking habits or other food sources)?	Acrylamide	Evidence from animal studies have pointed out that acrylamide may be carcinogenic, mutagenic genotoxic, neurotoxic and have adverse effect on fetal growth. See also HBM studies listed above.	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. Actions: - generate new HBM data for EU populations where there is a gap that can be considered in further HBM programs. - 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. -data analysis to identify current exposures levels, temporal and geographical trends and data gaps. -collection, comparison and evaluation of existing data and integration into IPCheM. **Relevant WP: WP7 WP8 WP10 WP11 WP13**

Policy related questions	Substance	Available knowledge	Knowledge gaps and activities needed
6) Is there a possible mixture of effect between acrylamide and other carcinogens?	Acrylamide		There is limited knowledge for a mixture of effect between acrylamide and other carcinogens, particularly dietary carcinogens Actions: -To perform investigations for the understanding of mixture of effects between acrylamide and dietary carcinogens e.g benzopyrene. **Relevant WP: WP15**
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?	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.	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. Action: -To evaluate whether the EU regulations had an impact on the reduction of exposure level of acrylamide and weather other restrictions should be implemented for the food industry. **Relevant WP: WP10**

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6 Prioritized substance group: Aprotic solvents

6.1 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 prioritization 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).

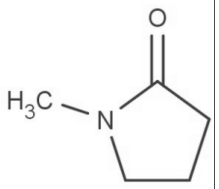
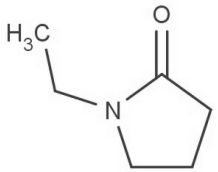
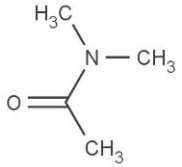
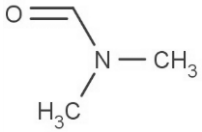
So, according to this proposal, the priority group of substances can be rephrased as “reprotoxic aprotic solvents”.

6.1.1 Hazardous properties

6.1.1.1 General characterisation

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

Table 6.1: Identification of HBM4EU prioritised reprotoxic aprotic solvents

Name	Abbre-viation	CAS number	EC number	Formula	Structural formula	Harmonised classification acc. to CLP
1-methyl-2-pyrrolidone	NMP	872-50-4	212-828-1	C_5H_9NO		Repr. 1B, H360D STOT SE 3, H335 (C ≥ 10 %) Eye Irrit. 2, H319 Skin Irrit. 2, H315
1-ethylpyrrolidin-2-one	NEP	2687-91-4	220-250-6	$C_6H_{11}NO$		Repr. 1B, H360D
N,N-dimethylacetamide	DMAC	127-19-5	204-826-4	C_4H_9NO		Repr. 1B, H360D Acute Tox. 4, H332 Acute Tox. 4, H312
N,N-dimethylformamide	DMF	68-12-2	200-679-5	$HCON(CH3)_2$		Repr. 1B, H360D Acute Tox. 4, H332 Acute Tox. 4, H312 Eye Irrit. 2, H319

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

6.1.1.2 Human toxicological information

A number of key animal studies providing information on reproductive toxicity of selected aprotic solvents are summarized 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 sternebrae 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 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 sternebrae 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 diferences 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 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 sensitization 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 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 recognized 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 recognized (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).

6.1.2 Exposure characteristics

6.1.2.1 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

(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.

6.1.2.2 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 pages[2].

6.1.2.3 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, neutrallisation 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 known consumer use conditions. At the workplace, however, inhalation and dermal uptake can be

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

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).

6.1.2.4 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 derivatization (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).

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 analyzed 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 µmol/l for groups 1, 2 and 3, respectively, in plasma and 420, 360 and 62 µmol/l in urine adjusted for density.

For 2-hydroxy-N-methylsuccinimide (2-HMSI), the maximal concentration was 5.4, 4.5, and 1.3 µmol/l for groups 1, 2 and 3, in plasma, respectively, and 110, 82 and 19 µmol/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, derivatized, and analyzed 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 µmol/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 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/m^3 (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.

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 ($P < .0001$, $r2 = .54$) 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 minimize 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. 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/m^3.

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. 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/m^3 (range < 0.15-46.9 mg/m^3).

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.

6.1.2.5 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**

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].

[3] https://www.ncbi.nlm.nih.gov/pubmed/23257108

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).

6.1.3 Policy relevance

6.1.3.1 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 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).

[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

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 2018[7].

In addition, DMF is considered as Category 2A substance with respect to carcinogenicity according to IARC[8] 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 categorizations 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.

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-TWA[10]) of **10 ppm** (**40 mg/m³**), a short-term exposure limit (TLV-STEL[11]) 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/119[12].

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 10/01/1990[14]).

6.1.3.2 Upcoming regulation

NEP, DMF and DMAC are likely to be considered for restriction in the future. ECHA RAC started to evaluate the DMF restriction proposal in November 2018.

[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
[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

6.1.4 Technical aspects

6.1.4.1 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) (Fig. 1).

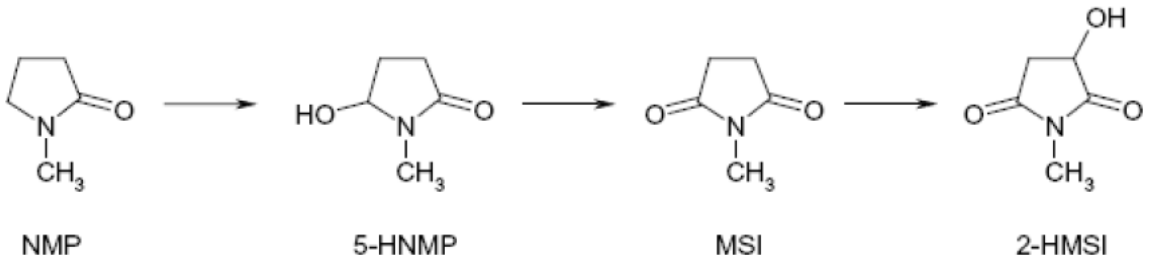


Figure 6.1: Proposed metabolism of NMP (Akesson et al., 1997; Carnerup et al., 2005).

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).

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). According to French National institute of research and security (INRS) and its Biotox database[15], 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).

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

6.1.4.2 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

[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).

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.

6.1.5 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 authorization.

[16] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5886881/

6.2 Categorization of Substances

Table 6.2: Substances included in the substance group, listed according to availablitity 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 authorization, restricted under Annex XVII **CLP**: harmonized classification Repr. 1B, H360D
	DMF	N,N-dimethylformamide	68-12-2	**REACH**: SVHC, included in the candidate list for authorization **CLP**: harmonized 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 authorization **CLP**: harmonized 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**: harmonized classification Repr. 1B, H360D

6.3 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?

6.4 Research Activities to be undertaken

Table 6.3: 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	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.	Very general knowledge about releases to environment – the related information should be gathered. 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. No information on content in widely used consumers` products – investigations should be arranged. Information on indoor pollution is lacking – special investigations should be arranged. 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. 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. Information on REACH restriction success is lacking – such investigations shall be done after the transitional period. Association between exposure of general population and lifestyle and consumption patterns is unclear – special investigations shall be arranged.
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.	Very general knowledge about releases to environment – the related information should be gathered. 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. No information on content in widely used consumers` products – investigations should be arranged. Information on indoor pollution is lacking – special investigations should be arranged. 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. 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. Information on success in relation to prohibition in cosmetic products is unclear - such investigations shall be done. Association between exposure of general population and lifestyle and consumption patterns is unclear – special investigations shall be arranged.

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.	Very general knowledge about releases to environment – the related information should be gathered. 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. No information on content in widely used consumers` products – investigations should be arranged. Information on indoor pollution is lacking – special investigations should be arranged. 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. 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. Information on success in relation to prohibition in cosmetic products is unclear - such investigations shall be done. Association between exposure of general population and lifestyle and consumption patterns is unclear – special investigations shall be arranged.
1, 2, 3, 4, 5, 6, 7, 8	NEP	Toxicological information. Established biomarkers of exposure and HBM values. Analytical methods in place. Notion on the most significant exposure route.	Very general knowledge about releases to environment – the related information should be gathered. 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. No information on content in widely used consumers` products – investigations should be arranged. Information on indoor pollution is lacking – special investigations should be arranged. 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. 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. Association between exposure of general population and lifestyle and consumption patterns is unclear – special investigations shall be arranged.

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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7 Prioritized substance group: Arsenic

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7.1 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.

7.1.1 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].

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).

7.1.1.1 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 metabolized 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).

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 characterization 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].

7.1.2 Exposure characteristics

7.1.2.1 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 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/l1, rising to 100–5000 g/l in areas of sulfide mineralization 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 absorber 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/m^3. According to USEPA the estimated average national exposure in the U.S. is at 6 ng As/m^3. 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 m^3 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 absorber.Typical arsenic levels for the European region are currently quoted as being between 0.2 and 1.5 ng/m^3 in rural areas, 0.5 and 3 ng/m^3 in urban areas and no more than 50 ng/m^3 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 77.0mcg/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 cross 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, 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, standardized 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 summarized in a report from the World Health Organization (2015) and are shown in the table below.

Table 7.1: 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	
			Blood (ng/mL)	Urine		Urine (µg/g creat.)	references
				(µg/g creat.)	(µg/L)		
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

7.1.2.2 Health based guidance values available for HBM data

7.1.2.2.1 General population

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

Table 7.2: 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].

7.1.2.2.2 Occupational population

The following recommendations are available:

Table 7.3: Recommended Biological Limit Values (BLV) for occupational exposure

Organization	Biological Limit Value (BLV)	Reference
Germany/ Deutsche Forschungsgemeinschaft (DFG)	• 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	• 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 ofpersons 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]*

7.1.3 Policy relevance

7.1.3.1 European Policies

European legislations concerning arsenic are described below.

7.1.3.2 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 ($BMDL_{01}$ = 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.

7.1.3.3 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.

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	AsCl3	232-059-5	7784-34-1	yes		ppm	
Diarsenic trioxide	As2O3	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	Pb3(AsO4)2	222-979-5	3687-31-8	Yes, INACTIVE	1		
Calcium arsenate	Ca3(AsO4)2	231-904-5	7778-44-1	Yes, INACTIVE	1		
Tricopper arsenide	Cu3As	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)*

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.

7.1.4 Technical aspects

7.1.4.1 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 mineralization). 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 this 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.

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 harmonize 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 harmonize 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 characterization 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 realization 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.

7.1.5 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 recognized 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.

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. **I**t should be noted that this priority list is not a list of "most toxic" substances, but rather a prioritization 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 organized in the frame of HBM4EU in on November 20th 2017 as a "top substance of concern".

7.2 Categorization 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 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)

7.3 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 harmonized, 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?

7.4 Research Activities to be undertaken

Table 7.4: 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 - collection, comparison, evaluation and integration into IPChem - identification of knowledge gaps - prioritization 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 - 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
What are possible health effects resulting from chronic low exposure to arsenic from food consumption?	As	Human exposure and effects data are limited.	- 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.

Policy question	Substance	Available knowledge	Knowledge gaps and activities needed
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.	Preparation of a core study to assess: - (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.	Mapping of existing capacities - 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 harmonized, 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
How can HBM4EU results support European policy decisions?	As		- Identification of stakeholders - Mapping, prioritizing 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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8 Prioritized substance group: Diisocyanates

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8.1 Background Information

8.1.1 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-tolylidene 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.

Table 8.1: 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)*

8.1.2 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.

8.1.3 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 ≥ 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).

8.1.4 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 analyzed 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 sensitization, 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 continuos exposure pattern. The ability of adducts to detect occasional exposures to sensitizing 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).

8.1.5 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 sensitization 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 sensitization.

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. 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.

8.2 Categorization of Substances

Table 8.2: 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: Annex XVII of REACH
	-TDI			
C	HDI			
	IPDI			
	NDI			
D	XDI			
	TODI			
	HDMI			
	TMXDI			
	TRIDI			

8.3 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?

8.4 Research Activities to be undertaken

Table 8.3: 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	Action: 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.	Gaps: Exposure in SMEs using diisocyanates for different purposes? Sensitivity of the HBM methods at low exposure levels? Action: 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)	Sensitization capacity of diisocyanates known. Also some diisocyanates possibly carcinogenic.	Gaps: What are the risks at current exposure levels. Is there a cancer risk due to formation of respective diamines? Action: Risk assessment based on biomonitoring data taking into account both asthma and cancer risks. This may need also PBPK modelling data.

8.5 References

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9 Prioritized substance group: Lead

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9.1 Background information

9.1.1 Hazardous properties

Lead is a soft, silvery grey metal. It is highly resistant to corrosion, but is soluble in nitric and hot sulphate acids. Solubility in water varies: lead sulphide and lead oxides are purely soluble while nitrate, chlorate and chloride salts are reasonably soluble in cold water. Lead also forms salts with organic acids as lactic and acetic acids, and stable organic compounds such as tetraethyl lead and tetramethyl lead.

Although lead and its organic compounds occur (or used to occur) in various man-made substances like petrol additives (tetraethyl- and tetramethyl lead), or lead-based paints (lead(II) chromate - „chrome yellow", lead (II,IV) oxide – „red lead", lead carbonate – „white lead"), a considerable proportion of human exposure is also resulted from inorganic lead or lead salts (lead pipes and solder in plumbing systems, lead-soldered food cans, batteries, etc.). Independently of their original form the toxicity of lead compounds is determined by their ionic lead content (IARC, 2006), therefore human biomonitoring of lead exposure concentrates on measuring inorganic lead in human biological materials.

9.1.1.1 Absorption and distribution

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). (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 (Jakubowski, 2012).

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 mobilized by certain physiological processes like pregnancy or other factors.

9.1.1.2 Health effects

9.1.1.2.1 General overview of health effects

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) and organic lead compounds were not classifiable as to their carcinogenicity to humans (Group 3) (IARC, 2006).

Epidemiological evidence indicated **cancers** of the stomach, lung, kidney, and brain in workers exposed to inorganic lead, but not in all studies.

Genetic susceptibility to lead exposure related to ALAD gene polymorphism has been indicated by some but not all studies (IARC, 2014).

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.

Lead can easily cross the placental barrier, therefore can readily enter the bloodstream of the foetus. Since Pb can also pass the blood brain barrier, neurological development is of great concern when prenatal exposure to lead occurs (Baeyens et al., 2014). There is also a potential link between blood lead level and increase of blood pressure in pregnant women at low level exposure (Wells et al., 2011)

A 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).

Lead is known to affect several enzymatic reactions critical in haem synthesis resulting in ***anaemia***. (EHC, 1995)

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).

9.1.1.2.2 Acute clinical toxicity

Intense, acute, high-dose exposure to lead can cause symptomatic poisoning in children. It is characterized 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).

9.1.1.2.3 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

Anaemia is the classic clinical manifestation of lead toxicity in erythrocytes. 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).

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).

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).

9.1.2 Exposure characteristics

9.1.2.1 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).

9.1.2.2 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/m^3, in suburban ones 0.33 µg/m^3 and in rural areas 0.1 µg/m^3). 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).

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.

9.1.2.3 Availability of HBM data

Surveys measuring blood lead levels in the general population have been conducted in several countries since the early 1980-ies. 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 7 countries (Belgium, Germany, Denmark, Kosovo, Poland, Slovenia and Sweden) were found from where blood lead levels measured during the past 5 years were available.

Table 9.1: 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	
		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	Fierens et al. (2016)
		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	Hruba et al. (2012)
Czech Republic	Prague	7-14 years	8	2007-2008	GM: 15.5; Range:12.0-22.0	Hruba 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 Foraeldre/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	

Country	Study	Population studied	N	Year of sampling	PbB (µg/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 ±19 (Range: 5-163)	Kutllovci-Zogaj et al (2014)
	Shtime (control)	6-12 years	53	? 2012-2014	AM: 23 ±7 (Range: 12-52)	
	Mitrovica	kindergarten	31	? 2012-2014	AM: 38 ±13 (Range: 22-77)	
Poland	Upper Silesia	3-18 years	4,882	1999-2013	? (in Abstract not available)	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 ± 13.59	Szkup-Jabłońska et al. (2012)
	Piekary Śląskie (Silesia)	3 – 6 year	678	2013	GM: 24.7 ± 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)

Country	Study	Population studied	N	Year of sampling	PbB (µg/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)
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	

9.1.2.4 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 recognizing 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 led** 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), and based on the results of GerES III and IV, in combination with current data from the German Environmental Specimen Bank, the following statistically derived ***reference levels*** were identified: 4 **µg/dL** for adult men, 3 **µg/dL** for adult women and **3.5 µg/dL** for children (UBA, 2018).

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: 5 µg/dL in the case of developmental neurotoxicity, 6.3 µg/dL for chronic kidney disease and 15 µg/dL for elevated systolic blood pressure (EFSA, 2010).

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

9.1.3 Policy relevance

9.1.3.1 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/m^3 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 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.

9.1.4 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 methods include graphite furnace AAS (GFAAS, also known as electrothermal AAS), ASV, and ICP-MS. Information on laboratory performance (i.e., accuracy and precision) from external and internal quality control data should be provided.

9.1.5 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 marginalized 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 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)

9.2 Categorization of Substances

Table 9.2: 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)

9.3 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?

9.4 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 harmonized within partner countries, please indicate this too.

Table 9.3: 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 showing interest in participation, 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 in small groups of children. Detailed description of sampling circumstances should be provided..

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10 Prioritized substance group: Mercury and its organic compounds

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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.

10.1.1 Hazardous properties

10.1.1.1 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 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 harmonized 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).

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).

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.

10.1.1.2 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).

10.1.2 Exposure characteristics

10.1.2.1 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 characterized 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 revolatilization from land and surface water. This emission, deposition and revolatilization 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 pray 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.).

10.1.2.2 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 sensitizer 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.
- **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, Jaćimović, Dizdarevič, & Horvat, 2013).

Human exposure to mercury begins at the time of conception and continuous 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 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 & Sulliva, 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).

10.1.2.3 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).

DEMOCOPHES, carried out in 2010-2012, was the first Europe-wide harmonized 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 scalp-hair samples. The mercury concentrations found at European level, were Mean=0.14 µg/g, P_{90}=0.82 µg/g for children and Mean=0.22 µg/g, P_{90}=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 summarized 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.

Table 10.1: 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 analyzed 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 analyzed 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	
	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)	-	

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)			
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	-	
	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)

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=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, Etchevers, & Fillol, 2012)
		Children Age: 3–17 y N=1364	-	-	GM=0.37 P95=1.20	-	-	
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
	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	-	-	

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 IV (2003-2006)	Children Age: 3-14 y N=1552	GM=0.23 P_{90}=0.3	-	GM<0.1 P_{90}=0.3	-	-	reference and HBM values derived by the German Human Biomonitoring Commission", 2012)
	GerES V	Children and adolescens 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)	-	

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)			
Italy	PROBE (2008-2010)	Adolescents Age: 13–15 y N=252	GM=0.84 P95=3.55	-	-	-	-	(Pino, Amato, Alimonti, Mattei, & Bocca, 2012)
	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)

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)	-	
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)

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)			
	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)
		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)	-	

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)			
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.)
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

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 10.2: 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	Faroes: 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)
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)

Country	Birth Cohort	Metals	Enrollment Period	No. of Children at Birth	Ref.
Croatia	Implementation of human biomonitoring survey of prenatal exposure to mercury in two Croatian regions using the standardized 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 Francaise 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 summarized in the tables below.

Table 10.3: 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

Table 10.4: 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

10.1.2.4 Health based guidance values available for HBM data

10.1.2.4.1 General population

The following table summarizes 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 10.5: 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

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).

10.1.2.5 Occupational population

The following recommendations are available:

Table 10.6: Recommended Biological Limit Values (BLV) for occupational exposure

Organization	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)
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)

10.1.3 Policy relevance

10.1.3.1 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.

10.1.3.2 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 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 Commisssion, 2007).

The European Food Safety Authority (EFSA) and national food safety authorities provide advice on fish consumption in an attempt to minimize 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 minimizing 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 realize 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 Sates 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).

10.1.3.3 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 harmonized 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 §10.1.1). 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).

10.1.3.4 Environment

Regulation (EU) 2017/852 transposes in the European Union the obligations under the Minamata Convention on Mercury (see §10.1.3.7). 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, stabilized 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 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 authorized 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.

10.1.3.5 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.

10.1.3.6 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 agents that are present at the workplace or as a result of any work activity involving chemical agents. *Directive 2009/161/EU26* 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/m^3).

10.1.3.7 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 organizations, 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 harmonized 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 as well as trends in levels of mercury and mercury compounds observed in biotic media and vulnerable populations.

10.1.4 Technical aspects

10.1.4.1 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 analyze 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 (ISCIII)) 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 analysis is preferable for a comprehensive assessment of the type and magnitude of the exposure.
- **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 Višnjevec, 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).

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 harmonized 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 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 hypothesized exposure.

10.1.4.2 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 harmonize 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 harmonization 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.

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 characterization 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 realization 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.

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

10.1.5 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 organizations recognize 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' organizations in Europe, with around 140 member-organizations 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 organizations in more than 100 countries
- "Zero Mercury" campaign of the Zero Mercury Working Group (ZMWG) (Zero Mercury Working Group (ZMWG) , n.d.).
  The Zero Mercury Working Group (ZMWG) is an international coalition of over 95 public-interest environmental and health non-governmental organizations 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 organization 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 organized 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.

10.2 Categorization 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 harmonized 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.

Table 10.7: 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	Abbreviation/ Acronym	Systematic name	CAS No.	Regulation
A	Hg	Mercury	7439-97-6	See §10.1.3*
B	MeHg, CH_3-Hg	Methylmercury	22967-92-6	See §10.1.3*

* *Section §10.1.3 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*.

10.3 Policy-related questions

Section §10.1.3 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. 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?
2. What toxicological data on mercury (and its species) are available and what new data are needed to answer policy-related questions?
3. How can harmonized, validated and comparable information be collected to support and evaluate current policies?
4. How can information be exchanged regarding mercury / mercury compounds?
5. How can transfer of knowledge & technology be facilitated to support current policies?
6. Which populations remain vulnerable to health impacts from mercury exposure and how can they be protected?
7. 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)?
8. How effective are current policies (including the EU's Strategy on Mercury and the Minamata Convention, which was ratified by the EU and Member States) in reducing human exposure to mercury in Europe? Are these policies implemented effectively?
9. At what level of exposure to different mercury species and to total mercury are health effects likely to occur?
10. What are the safe levels of mercury species in fish and other food?

11. What are possible health effects resulting from chronic low exposure to mercury and its organic compounds (such as from food consumption and dental amalgams)?
12. What is the safe intake level for methyl and inorganic mercury that is without any appreciable health risk in the general European population?
13. What factors make people more susceptible to the development of health effects due to mercury exposure?
14. What are cumulative risks from concurrent exposure to other chemicals (mainly other metals and other reprotoxic substances)?
15. 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 (also see below)?
16. 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?
17. How can HBM4EU results support policy decisions at EFSA and ECHA?
18. How can occupational exposure policy and other policy initiatives be better coordinated?
19. How should occupational exposure be assessed when there is a previous exposure of the worker from other sources and since birth or childhood?

10.4 Research Activities to be undertaken

Table 10.8: Listing of research activities to be carried out to answer the policy questions

Policy question	Substance	Available knowledge	Knowledge gaps and activities needed
1, 4 § 10.1.3.7	Mercury / methylmercury / inorganic compounds	See section § **Error! Reference source not found.**	Mapping and / or updating existing biomonitoring / exposure data - collection, comparison, evaluation and integration into IPChem (WP7,8,9,10) - identification of knowledge gaps - prioritization of research needs
2 § 10.1.3.7	Mercury / methylmercury / inorganic compounds	See sections § **Error! Reference source not found.**, § **Error! Reference source not found.**, § 10.1.2.4	- Mapping / updating existing toxicological data - Development of PBPK model for mercury for better risk assessment (WP12)
3 § 10.1.3.7	Mercury / methylmercury / inorganic compounds	See section § 10.1.4	Mapping of existing capacities - best-suited biomarkers & matrices to assess (species-specific) exposure and links to adverse health-effects - 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 - guidelines for statistical analysis in line with the data analysis / management plan
3, 4, 8 § 10.1.3.7	Mercury / methylmercury / inorganic compounds	See section § 10.1.5	- Identification of stakeholders - Mapping, prioritizing and addressing stakeholder needs, starting with policy makers and scientists
9, 10	Mercury / methylmercury / inorganic compounds	See section § **Error! Reference source not found.**	- Use of existing data to assess the determinants of exposure, including geographic variations and their causes (e.g. environmental exposures, diet)

Policy question	Substance	Available knowledge	Knowledge gaps and activities needed
6, 7, 10, 11	Mercury / methylmercury	See section § 10.1.2	- 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) - Preparation of a core study to assess: (a) the current 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 (with representative data on sensitive population groups (e.g. high seafood consumers with distinction of populations consuming predator fish from those with low/no consumption of such fish, such as Arctic, Southern and Northern European populations) but also low-risk populations for comparison (b) the contributions of different sources (dietary, including different species of sea-food, dental amalgams, environmental, contaminated sites) to the body burden, with the aim to elaborate HBM threshold levels for Europe and safe upper limits for different types of foodstuff
3, 4, 5, 8 § 10.1.3.7		See section § **Error! Reference source not found.**	- Establish permanent European mercury biomonitoring as long-term support of global mercury policies - Emphasis on knowledge transfer to enable new, quality-assured, comparable data in countries, which ratified the Minamata convention through the established procedures at EU level. - Development of training modules - Organization of hands-on training for interested countries

Policy question	Substance	Available knowledge	Knowledge gaps and activities needed
12, 15, 16, 17		See section § 10.1.3.2 10.1.3.1(Food Safety)	- Support the development of advice with dietary recommendations to European stakeholders (consumers, policy makers, health practitioners), in cooperation with EFSA / ECHA, considering the different types of foodstuff in different parts of the EI, the toxicity and occurrence of 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 - Preparation of an inventory of current relevant national strategies in European countries - Development of targeted communication materials for different stakeholders
13, 14		See section § 10.1.3.7	- Explore the possible use of existing cohorts for the investigation of the adverse health effects due to chronic exposure to low levels of methylmercury and other mercury species (this exposure is the most relevant for Europeans), including the identification and possibly validation of markers of susceptibility

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11 Prioritized substance group: Mycotoxins

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11.1 Background information

Following a prioritization strategy described in Deliverable 4.3, mycotoxins were selected as a prioritized 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).

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11.1.1 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). In the coming decades climate change is expected to impact fungal growth and agricultural practices (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, 2018).

DON and FB1 were prioritized in the 2nd round of substance priorisation 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, 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 itsacetylated 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; Solfrizzo et al., 2014; Alassane-Kpembi et al, 2016; Assunçao 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, 2016; Martins et al, 2018), in biological samples from general population (Heyndrickx, 2015; Vidal et al, 2016; Brera et al, 2015) 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; Puntscher et al, 2018). Other authors also refer the possible interactions between environment and food contaminants, cadmium and deoxynivalenol, in different target organs (Le TH et al, 2018).

11.1.2 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).

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, metabolized and rapidly excreted through urine as shown recently by a human intervention study after exposure to DON and DON-3-glucoside (Vidal et al, 2018b). 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. 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, 2012b), Belgium (Huybrechts et al, 2014) and Germany (Gerding et al, 2014).

Females and males show different patterns of exposure levels, and human exposure to DON also shows some geographical differences (Cheng et al, 2017; Vidal et al, 2018b). 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) and Germany (Gerding et al, 2014). 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).

11.1.3 Policy relevance

In Europe, the European Commission (EU) has introduced comprehensive mycotoxin regulations for food to facilitate world trade and protect consumer´s health (Cheli et al., 2014). 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 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 harmonized 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).

11.1.4 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, 2018b). 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 synthesized mycotoxin-standards (Ediage et al, 2012; Warth et al, 2013). These analytical developments permitted the scientific community to find strong correlations between the sum of urinary DON and its glucuronides (Vidal et al, 2018b). 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, 2018b; 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 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).

11.1.5 Societal concern

It has been well recognized for many years that large economical losses occur worldwide owing to the mycotoxin contamination in agricultural products as recently summarized 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.

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 (Assunçao et al, 2016).

11.2 Categorization of Substances

Table 11.1: Substances included in the mycotoxins group, DON and FB1, listed according to availability of toxicology and human biomarker data, in category C substances*

**HBM scarcely exists, efforts to develop an anlytical method to obtain relevant HBM results need to be done, hazardous proprieties 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.*

Category	Designation (Abbreviation/ Acronym)	Systematic name (IUPAC name?)	CAS No.	Regulation
C	Deoxynivalenol (DON)	trichothec-9-en-8-one, 12, 13-epoxy-3,7,15-trihydroxy-, (3α,7α)	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-dimethylicosan-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

11.3 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 harmonized analytical methods to assess the selected mycotoxin exposure biomarkers?
2. What are the current exposure levels of the European population to the selected mycotoxins? Are there exposure data for other mycotoxins?
3. Does the exposure to mycotoxins differ among countries/EU geographical regions and different population groups? Which are the main factors related with these differences (age, gender, occupational settings, geographic localisation, season/year)?
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. Are there exposure models and toxicokinetics data for mycotoxins? Which are their limitations?
6. Is the risk associated with human exposure to these mycotoxins characterized? Are there health impact assessment studies? Is it possible to set a HBGV for mycotoxins in biological samples?
7. Does the aggregate exposure to mycotoxins/other food contaminants contribute to combined effects? What are the knowledge gaps for risk assessment?
8. Which are the key-events that determine the long-term health effects from low-dose continuous exposure to the target mycotoxins? Which are the health effects associated with high short-term exposure by inhalation (occupational exposure)?
9. Which are the most reliable and meaningful effect biomarkers for single and combined effects?
10. Are there mycotoxins (including metabolites masked and/or other modified forms) besides those covered by the current risk assessment, which could be potentially relevant concerning their (co-) occurrence and toxicological properties?

11.4 Research activities to be undertaken

Table 11.2: 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 harmonized analytical methods to assess mycotoxin exposure biomarkers?	mycotoxins	Analytical methods for DON and its glucuronides as well as FB1–4 are mainly based on MS. However, commercial sources for DON glucuronide standards are scarce and no certified reference materials are available for urinary DON biomarkers 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.	G: Current analytical methods, harmonized methods, reference materials, proficiency tests, expert laboratories A: 1. Identify across Europe the analytical capacity for determination of multiple biomarkers of exposure, availability of reference materials and standards; best biomarkers, matrices and methods (Y3) WP9 2. Promote training and harmonization on analysis of selected mycotoxins biomarkers including an inter-laboratorial assay (Y3, 4) WP2, WP9 3. Identify expert laboratories to conduct the inter-laboratorial trial (Y3) WP9 4. Elaboration of SOP for trial assay (Y3) WP9 5. Extension of qualified laboratories by introduction of HBM specialised laboratories (Y3,4) 6. Identify quality assurance requirements (Y3, 4) WP9 7. Identify needs and gaps.
2. What are 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 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 including vulnerable populations as children, special diet, pregnant women) and workers. A: 1. Perform an inventory survey on FB1 data before intiation of a large scale QA and monitoring activities to evaluate the percentage of left-censored date available. 2. Create a database for mycotoxin exposure using HBM data from different EU countries (gathered by national hubs) including mycotoxin identification, population group and ages, routes of exposure and HBM data. Collect, harmonize, compare data from different population groups available and evaluate (Y3, Y4). WP7, WP8, WP10 3. Integrate into IPChem (Y4). WP10 4. Identify needs (WP4) and gaps.WP7

Policy question	Substance	Available knowledge	Knowledge gaps (G) and activities needed (A)
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 localization, season/year)	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, diet, occupational setting, location, in EU A: 1.Identify risk groups, including highly exposed, vulnerable and hotspots in Europe (Y3,Y4) WP10 2. Statistical analysis (Y3). 3. Identify significant differences between analysed groups (Y4). WP10 4. Identify needs and gaps
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	More than half of all worldwide agricultural samples contain DON and FUM (Biomin Mycotoxin Survey). 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).	G: Analysis of trends on HBM mycotoxin exposure A: 1.Identify possible temporal and geographic trends related to HBM mycotoxin exposure taking seasonal variation into account (Y4) WP10 2. Evaluate significant differences (Y4) WP10 3. Identify possible reasons for the differences founded (Y4) 4.Identify needs and gaps.
5. Are there exposure models and toxicokinetics data for mycotoxins and which are their limitations?	mycotoxins	DON and its metabolite DON-3-glucoside were absorbed, distributed, metabolized and rapidly excreted through urine as shown recently by a 1st human intervention study after exposure to DON and DON-3-glucoside. 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).	G: Exposure models and toxicokinetics in humans A: 1. Explore the possibility of applying the previously developed toxicokinetic models to DON and FB1 (Y3) 2. Determine exposure levels from HBM databases and available literature through reverse dosimetry models (Y4) WP12 3. Identify needs and gaps

Policy question	Substance	Available knowledge	Knowledge gaps (G) and activities needed (A)
6. Is the risk associated with human exposure to these mycotoxins characterized? Are there health impact assessment studies? Is it possible to set a HBGV for mycotoxins in biological samples?	mycotoxins	The estimated mean chronic dietary exposure 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. Little if any work has been done in estimating the burden of human disease caused by exposure to the dietary mycotoxins. The only studies available are related to aflatoxin B1 (Wu et al, 2014; Assunçao et al, 2018b).	G: Risk characterisation and health impact assessment (HIA) A: 1. Identify available estimates of human exposure via biomarkers (Y3,4); 2. collect toxicological data (Y3,4) 3. If possible, establish HBGV values for mycotoxins in biological samples (Y4) (WP5) 4. From risk assessment to health impact assessment: trying to derive the consequences of human exposure to mycotoxins using epidemiological data (e.g. incidence of disease, age of onset of disease and its evolution) and data gathered on human exposure studies (e.g. DALYs) (Y5) WP5, WP11 5. Identify needs and gaps.
7. Does the aggregate exposure to mycotoxins/other food contaminants contribute to combined effects? What are the knowledge gaps for risk assessment?	mycotoxins	Co-occurrence of DON or FB1 and other mycotoxins has been widely reported and human aggregated exposure to mycotoxins and other food contaminants is likely to occur	G: Lack of an inventory of exposure to DON or FB1 and other mycotoxins/other food contaminants in EU and potential interactive effects A 1. Identify main mycotoxin/other food contaminants mixtures from available HBM data (biomarkers and routes of exposure); (Y3) WP15 2. Compare available HBM mixtures data over EU countries, look for significant differences and trends (Y4) WP15, WP10? 3. Assess common endpoints, determine whether the additive model is adequate to describe mycotoxins/other food contaminants combined effects; assess if this is dependent of mode of action or the target organ toxicity (Y4) WP15 4. Identify needs and gaps

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?Which are the health effects associated with short-term high exposure by inhalation (occupational exposure)?	mycotoxins	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. 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	G: Several health effects known and mechanistic data available but AOP for DON and FB1 lacking A. 1. Identify for DON and FB1 the health effect for which a AOP might be developed, e.g. immunotoxicity for DON and liver toxicity for FB1 (Y3) 2. Disclose the key-events for the effects referred in 1. in order to contribute to AOPs development (Y3, Y4) WP13 3. Identify needs and gaps
9. Which are the most reliable and meaningful effect biomarkers for single and combined effects?	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	G: Limited information on available biomarkers of effects A:1. Identify available targeted and untargeted biomarkers of effect for the selected mycotoxins (Y3, Y4) WP14 2. Identify biomarkers of effect related to interactive effects of mixtures (Y3, Y4) 3. Identify needs and gaps
10. Are there mycotoxins beside those currently covered by the risk assessment, which could be potentially relevant concerning their (co-)occurrence and toxicological properties?	mycotoxins	An increasing number of studies are paying attention to mixtures involving the “emerging” toxins (enniantins, beauvericin, Alternaria toxin, etc.).	G: Co-occurring forms (emergent mycotoxins) with potential toxicity and health impact that are not covered in risk assessment A1. Bibliography search (Y3,4) WP16 2. Identify most relevant co-occurring forms other than those already covered, to refine human risk assessmen (Y3,4,).WP16, WP5 3. Identify needs and gaps.

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12 Prioritized substance group: Pesticides

Pyrethroids (group), chlorpyrifos, dimethoate, glyphosate (including the co-formulant POE-tallow amine) and fipronil (D.4.5)

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12.1 Background Information

12.1.1 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 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).

12.1.1.1 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 therefor 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 depolarization 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

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 internalizing 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.

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).

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) 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)

12.1.1.2 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 disrupts 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 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 is the most used OP in the EU 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 at 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 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.

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 T_4 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.

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 immunetoxicity

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).

12.1.1.3 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 immunetoxicity

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 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 recognized 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.

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, zink, 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.

12.1.1.4 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 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.

12.1.1.5 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 emphasized 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).

12.1.1.6 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 marked (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 recognized, 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).

12.1.2 Exposure characteristics

12.1.2.1 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 therefor 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.

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 (Victora 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 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 characterize 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.

12.1.2.2 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).

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 therefor 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 metabolized (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).

12.1.2.3 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.

12.1.2.4 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 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

pplication 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 assesses to presents 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 therefor 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.

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 characterize 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.

12.1.2.5 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).

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 µg/kg/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 characterize 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.

12.1.2.6 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 stablished. 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	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 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 Reisfeld 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).

12.1.3 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.

12.1.4 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 therefor this matrix is not considered relevant. Chlorpyriphos 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. Harmonization of the methods within partner countries might be necessary.

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 analyzed 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) 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.

12.1.5 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 characterization 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 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.

12.2 Categorization of Substances

Table 12.1: 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
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 (JMPR 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

Cat.	Abbrev. / Acronym	Systematic name	CAS No.	Classification (EC1272/2008) and thresholds	Regulation
B		Zeta-cypermethrin	52315-07-8 (same as cypermethrin)	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	39515-40-7		Under review as BP T18
B		d-Allethrin	231937-89-6		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		Under review as BP T18
B		Empenthrin	54406-48-3		Under review as BP T18
B		Epsilon-momfluorothrin	1065124-65-3		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 (JMPR 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		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)
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		Approved as BP T18 (Dir. 2010/71/EU)
B		Prallethrin	23031-36-9		Under review as PB T18.

Cat.	Abbrev. / Acronym	Systematic name	CAS No.	Classification (EC1272/2008) and thresholds	Regulation
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		Not approved as PPP (2002/2076) Under review as BP T18
B		Transfluthrin	118712-89-3		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
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).
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).

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.	Appoved as PPP Reg. (EU) 2017/2324, Reg. (EU) No 540/2011 2016/1056, Reg. (EU) 2016/1313)
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

12.3 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?

12.4 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 harmonized within partner countries, please indicate this too.

Table 12.2: 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)	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). 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. 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. A method exist to measure some pyrethroids (total and some specific) and chlorpyrifos simultaneously. 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.	Activities: 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 harmonized 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).

Policy question	Substance	Available knowledge	Knowledge gaps and activities needed
	Cat C (dimethoate, fipronil, and POEA)	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) 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. 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.	Activities: 1. If considered relevant to include fipronil (Q2), urine will be the preferred matric, 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 considered relevant 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)	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. 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	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. Activities: 1. Collecting, comparison, and evaluation of existing biomonitoring data in the EU and integration into IPCHEM (Y3-Y4) (WP10) 2. Identify and prioritise knowledge and data gaps and related research needs (Y3) (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 (Y3-Y5) (WP8) 3. Data-analyses of time-trends and differences between countries and population groups, including identification of subpopulations with highest exposure levels (Y3-Y5) (WP10).

Policy question	Substance	Available knowledge	Knowledge gaps and activities needed
	Cat C (dimethoate, fipronil, and POE-tallow amine (POEA))	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. 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. 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 exposure sources.	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. Activities: 1. 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 (Y3-Y5) (WP8). 2. Prior to method development for POEA and fipronil (see Q2 below) it should be considered if there is a need to monitor these substances in human matrices (preferentially urine) at present (WP4). 3. If considered relevant, 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)

Policy question	Substance	Available knowledge	Knowledge gaps and activities needed
3. What are the main dietary sources of exposure across the member states?	Cat B and C (all substances)	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). 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.	Activities: 1. Analyse/model HBM data in relation to monitoring data on residues in food samples to 1) compare and complement exposure assessment performed by EFSA and 2) identify the major dietary exposure sources across member states (Y3-Y5) (WP12) 2. Perform a pilot study analysing selected pyrethroids and chlorpyrifos (parent compounds) in existing bio-banked milks samples (if available) (Y3-Y5) (WP9, WP8).

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)	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. 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. 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.	Activities: 1. Analysing the urine samples from the WP15/16 survey using the above-mentioned methods, will allow quantification of these pesticide metabolites (WP9) and 2. subsequent data analyses to compare the levels with those obtained from the alignment studies (WP10) and 3. comparison with the result obtained by the multi-target screening method (WP10) Data gap: Biocidal use of pyrethroids might be increasing in the EU but there is no HBM studies investigating this exposure situation. Activities: 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). 2. If a targeted study is not feasible it may be possible to get some information by analysing HBM data from the alignment studies 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 authorization and sale of biocidal products might also be included in data analysis of HBM data from the alignment to investigate exposure differences between member states alignment (Y3-Y5) (WP10).

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)	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. Further, workers employed in companies applying biocides (pyrethroids and/or fipronil) in dwellings and institutions might have high dermal and inhalation exposure.	Data gap: 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. Activities: A targeted study addressing occupational exposure levels is highly relevant (WP8). A possibility might be to extend the WP15/WP16 mixture survey to also include urine sampling of agricultural workers who mix and/or apply the pesticides (WP15, WP16, WP8)
6. Are the exposure levels of health-relevance/concern for vulnerable groups or high exposure population groups?		Most of the prioritised pesticides are neurotoxicants (OPs, pyrethroids, fipronil) and some also have ED or genotoxic/carcinogenic properties. The main health concerns are adverse effects on neurodevelopment and/or endocrine disturbances affecting reproduction, metabolism etc.	Activities: 1. Combining HBM data from EU studies, e.g, from birth cohort studies with health outcomes – if possible using meta-analysis (WP13) 2. Identify/suggest adverse outcome pathways (AOPs) for relevant health outcomes, including neurodevelopment (WP13). 3. Identify/suggest relevant effect biomarkers (WP14)
7. How can cumulative risks of pesticide mixtures on health outcomes be assessed and integrated in regulation?		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)	Input from WP15
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?			Comparison of HBM values with toxicologically derived guidance values (ADI values) and findings on associations with health outcomes in (Y3-Y5) (WP15) Input from WP5 and WP15
9. How can HBM data from HBM4EU feed into prioritisation of the pesticides for risk assessments and regulatory decision-making?			Input from WP5

Annex 1: HBM data on pyrethroids (3-PBA), OPs (DAPs), chlorpyrifos/chlorpyrifos-methyl (TCPY) and glyphosate

Table 12.3: HBM data on pyrethroid exposure based on urinary concentrations of the generic pyrethroid metabolite 3-PBA. The values represent volume-based concentrations (μg/L) in spot urine samples unless otherwise stated.

Study	Population	Sampling year	N	LOD	%>LOD	GM	50th pct (median)	95th 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)
Elfe, 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*	*μg/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)
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	Pregnant women	98-01	307			-	18.3	126.9*	Third trim, *90th pct	Berkowitz et al. (2003)

Study	Population	Sampling year	N	LOD	%>LOD	GM	50th pct (median)	95th pct	Remark	Ref
York, USA									Residential use	
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

Table 12.4: HBM data on OPs. Urinary concentrations of total dialkyl phosphate metabolites (ΣDAP; 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)	95th 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,	Children10-16 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 μg/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)
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

Table 12.5: HBM data on chlorpyrifos/chlorpyrifos-methyl. Urinary concentrations of the specific metabolite TCPY. The values represent volume-based concentrations (μg/L) in spot urine samples unless otherwise stated.

Study	population	Sampling year	LOD	%>LOD	N	GM	50th pct (median)	95th 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	Children10-16 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*	*μg/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*	*μg/g creatinine	Babina et al. (2012)
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

Table 12.6: HBM data on glyphosate and AMPA. Urinary concentrations (μg/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
	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

Reference	Country	Period	Population	N	Definition of average	Value average**	Calculation high values	High value**
	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

[a]*corrected for age, sex, smoking and urine density*

** *Gly = glyphosate*

Annex 2: HBM4EU– suggested pesticide biomarkers/metabolites and reference to established analytical methods

Pesticide group	Metabolite/biomarker (abbereviation)	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
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-methhyl-3-phenylbezoic acid (MPA)	115363-11-6	Bifenthrin	Urine	GC_MS, 0.04(Tao et al. 2013)	?

Pesticide group	Metabolite/biomarker (abbereviation)	Cas no	Parent pesticide/compound	Matrix	Analytical methods, reported LOD/LOQ in ng/ml (Reference)	Status
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	Chlorotrifluorovinylcyclo-propane carboxylic acid (CIF-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
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*

12.5 References

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13 Prioritized substance group: UV filters

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13.1 Background information

13.1.1 Hazardous properties

- **Benzophenone 3 (BP-3)** displays a low acute toxicity profile. It is not considered as being irritating to the skin and the eyes[1]. 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 orally[2]. BP-3 is on the Community Rolling Action Plan (CoRAP) list because of potential endocrine disruption[3]. BP-3 elicited anti-androgenic activity in a human breast carcinoma cell line[4] and interferes with functions of human sperm cells in vitro[5]. Critical effects are maternal and developmental toxicity[6]. In female mice, low dose exposure causes long-lasting alterations to mammary gland morphology and function[7]. Studies in rat primary cortical neuronal cultures and neuroblastoma cell lines showed decreased cell viability after BP-3 treatment at moderate concentrations[8].
  In a study on young men from Spain, there was a significant positive association between urinary BP-3 concentrations and serum FSH levels[9]. In male adolescents in the US, urinary BP-3 was associated with lower total testosterone[10]. 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[11]. In a study in healthy, premenopausal women, UV filter factors (BP-1, BP-3) were associated with decreased estradiol, FSH, and LH[12].
- **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 haematopoetic system, including rare histiocytic sarcomas. Available evidence supports that benzophenone is not genotoxic. Benzophenone meets the criteria for classification as carcinogenic in category 2[13]. Benzophenone may alter endocrine signalling through multiple effects on receptors.[4] Critical effects are liver and kidney effects.[6]
- **Benzophenone-1** (BP-1) is used as a UV filter, but is also the major 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 disruptors.[6]
  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.[11] In a study in healthy, premenopausal women, UV filter factors (BP-1, BP-3) were associated with decreased estradiol, FSH, and LH.[12]

- **Benzophenone-2** (BP-2) is a UV filter 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 iodide[14]. Both BP-2 and BP-3 were shown to exert uterotrophic effects and BP2 was shown to bind to estrogen receptors[15]. 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 LH, cholesterol levels, fat deposition, and an increased risk of endometriosis[16].
  In a study on exposure to UV filters and fertility, male partners' concentrations BP-2 was associated with reduced fecundity[17].
- **4-Methylbenzylidene camphor** (4-MBC) is found in cosmetics and in drinking water.[6]
  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 on rats found 4-MBC to have development toxicity.[6,18]
- **3-benzylidene camphor** (3-BC) - 3-BC is a potential endocrine disrupter. Experiments in vivo and in vitro revealed oestrogenic activity. In addition, 3-BC was found to interrupt sexual development and maturation in animal models.[14] 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 (ERα, ERβ, SRC-1 and PR (progesterone receptor)) has been shown to be altered in both males and females rats.[15]
- **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)[19].
- **4-methylbenzophenone** (4-MBP) is used in paints and varnishes, in food packaging but not in cosmetics.[5] 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 carcinogen[20].

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)

13.1.2 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 light.[6]
- 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 (in serum) of 19 hours [4].
- Human biomonitoring (HBM) data: pregnant women in US (California)[21], France[22], China[23], Israel[24], general public in Belgium[25], Denmark[26], and the US[27]. Data on exposure in children in available for the US[28], Denmark[29,30,31,32], China[33], Australia[34], Taiwan[35] and Germany[36] (GerES V, publication in preparation, for HBM4EU available data on 3 to 14 year old children and adolescents; young adults: 20-29 years, Environmental Specimen Bank).
- Several biomonitoring studies (including NHANES) have focused on BP-3.[27] 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 products[27].

13.1.3 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 products[37]. 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 preparations[38].
- According to the Scientific Committee on Consumer Safety, the use of 3-BC as a UV-filter in cosmetic products in a concentration up 2.0% is not safe[39].
- Benzophenone is approved as an additive in plastic food contact materials, with a specific migration limit of 0.6 mg/kg[40].
- 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.

13.1.4 Technical aspects

- BP-3 can be directly measured and quantified in urine in HBM studies. Benzophenones including BP-1 and BP-3 can be measured using an on-line LC/LC-MS/MS method for the simultaneous determination of nine parabens and seven environmental phenols in urine[41]. 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 methods[42].
- 4 – MBC urinary metabolites (3- (4-carboxybenzylidene) camphor and 3-(4-carboxybenzylidene)-6-hydroxycamphor) can be measured using gas chromatography high resolution mass spectrometry (GC-HRMS)[43].

- LC-MS/MS based methods have been developed in Germany[36] for simultaneous biomonitoring of nine parabens and seven environmental phenols including BP-3 and BP-1 and in Denmark[32] for simultaneous biomonitoring of nine UV filters in urine (BP, BP-1, BP-2, BP-3, 3-BC, 4-MBC, 4-HBP, 4-HBP, and 5-chloro-2- hydroxybenzophenone). However, urine might not be the preferred matrix for measurements of the most lipophilic UV filters such as 3-BC and 4-MBC.

13.1.5 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.

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 kinitics, metabolism, 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 criteria[44].

BP, BP-2 and BP-3 are on the SIN (“Substitute It Now”) list.

In addition, CHEMTrust nominated the group of benzophenones 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 (synonyme for BP-3). 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.***

It is also noteworthy that HBM studies showed that a majority of the populations were exposed to BP-3, and many of these studies cover year-round sample collection or winter time time sample collection.[29,32] Therefore, the major sources for BP-3 exposure might not be sunscreens.

Of note, due to reports on adverse effects of UV filters on coral reef, there is societal concern about ecological effects of suncreens.

13.2 Categorization of Substances

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 (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 categorize 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.

For the remaining substances, we propose to categorize 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.

13.3 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? (Sunscreens, cosmetics and personal care products, plastic and other food contact materials, textiles, furnitures and building materials and others)
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)
7. Was the restriction of BP-3 in cosmetics in the EU (September 2017) effective in reducing public exposure? Did exposure to other benzophenone or other UV filter compounds increase as a result?

13.4 Research Activities to be undertaken

Table 13.2: 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	Methods have been reported for BP-3 and three oxidative metabolites (2,4-dihydroxylbenzophenone, 2,2′-dihydroxy-4-methoxybenzophenone, and 2,3,4-trihydroxybenzophenone); and simultaneous measurement of 9 UV filters in urine	**WP9** - Are HBM methods to measure BP-3 / its metabolites and other benzophenones quality assured? Are levels of detection and quantification adequate? - Is there a need to develop new analytical methods?
2. What are current level of exposure of the EU population to benzophenone UV-filters?	Benzophenones, emphasis on BP-3	Benzophenones are likely to be increasingly detected in the general population in the EU, due to their extensive use in personal care products (sunscreens), food contact materials, and other products.	**WP7 & WP8; WP16** Systematic collection of available HBM data on benzophenones; Generation of data in targeted studies and from bio-banked samples if available. **WP10** Is existing exposure data sufficient to derive valid estimates for the exposure of the EU population? What data is needed to derive reference values?
3. Do the exposure levels differ significantly between the countries?	Benzophenones, emphasis on BP-3	Human biomonitoring data is scarce (only available for some countries with different population groups measured, e.g. France, Denmark)	**WP7 & WP8; WP10; WP16** Systematic collection of available HBM data on benzophenones; Generation of data in targeted studies and from bio-banked samples if available.
4. What are the main sources of exposure to benzophenones?	Benzophenones	The main sources of exposure to benzophenones are cosmetics and personal care products; food packaging materials; and other uses in consumer products	**WP10** What are major sources of exposure to benzophenones in the general population and in sub-groups? (per single substance and substance group) **WP12** Estimation of the contribution of different routes of exposure to the total exposure.
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	There is insufficient research to date to answer these questions; indications that exposure is higher in females due to increased use of personal care products and/ or cosmetics	**WP10** - Based on existing data, determine different exposure levels between: males/females, different age groups (depending on the data available) - In case occupational population data exists, determine different exposure levels in occupational populations in comparison with the general population **WP8** Targeted HBM studies on benzophenone exposure
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
7. Are potential health effects related to age and gender?	BP-3	The current research is not sufficient to answer this question	**WP10 & WP11** Epidemiological studies investigating endocrine effects **WP13** Investigate associations between exposure and health outcomes

Policy question	Substance	Available knowledge	Knowledge gaps and activities needed
8. How can cumulative risks of benzophenones and other UV filters be assessed for their health relevance? Are their additive (or other) effects relevant for regulation?	Benzophenones and other UV filters	The current research is not sufficient to answer these questions	**WP15** Cumulative risk assessment **WP5 & WP15** Assessing the feasibility of deriving an HBM health-based guidance value for combined UV-filter exposure
9. How can HBM4EU results feed into regulatory decisions and risk assessments (ECHA and EFSA)?	UV filters, specifically BP-3		**WP5** - Derivation of Health-based guidance values using HBM data for benzophenones **WP5** How can HBM data on benzophenones inform chemical risk assessment and management (exposure assessment, TDI evaluation)? What HBM data is needed to inform risk assessment and management? **WP12,13,14,15** Instruments to link health and exposure and to better estimate risks will be explored and their suitability in risk assessment and management will be evaluated (e.g. cumulative risk assessment)

Table 13.3: 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
Copenhagen University Hospital	Denmark	2013	Mother-child pairs	urine	BP-3	Frederiksen et al
Institute of Ruhr University Bochum	Germany	2014	Children and adults	urine	BP-1, BP-3, BP-8	Moos et al
University of Liege	Belgium	2014	Adults	Urine	BP-3	Dewalque et al.
University of Copenhagen	Denmark	2014	Children, adolescents, young men, and pregnant women (review)	Urine	BP-3	Frederiksen et al
Queensland	Australia	2015	Children and adults	Urine	BP-3	Heffernan et al.
Several universities	China	2015	Young children	Urine	BP, BP-1, BP-2, BP-3, BP-8, 4-HBP	Gao 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
University of Copenhagen	Denmark	2018	Mothers and fetus	Serum and urine	BP-1, BP-3, 4-MBP, 4-HBP	Krause et al

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