Ref. Ares(2022)4267188 - 09/06/2022

HORIZON2020 Programme
Contract No. 733032 HBM4EU

Human Biomonitoring in risk assessment: 4th set of examples on the use of HBM in risk assessments of HBM4EU priority chemicals

ANNEX to Deliverable Report

D5.11

WP5 – Translation of results into policy

Deadline: April 2022

Upload by Coordinator: 09 June 2022

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Risk assessment for acrylamide

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Acknowledgments

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Cathrine Thomsen, Line Småstuen Haug, and Amrit Kaur Sakhi (NIPH),

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and all HBM4EU aligned study and aggregated data owners for providing the data.

1.1 Summary

Acrylamide is an organic compound produced for different uses in the chemicals industry. It is also formed, when certain foods are prepared at temperatures above 120 °C in the absence of moisture. Acrylamide is detected in numerous baked or fried carbohydrate-rich foods, including frequently consumed foods in all population groups. In addition, acrylamide is present in tobacco smoke.

Acrylamide has been shown to have neurotoxic, carcinogenic, genotoxic and mutagenic effects (Muta. 1B, H340, Carc. 1B, H350 and STOT RE 1, H372** according to the Classification, Labelling and Packaging (CLP) regulation). It also has possible/suspected immunotoxic and developmental toxic effects, and adverse effects on the reproductive function in males (Repr. 2, H361f*** CLP classification). Biomarkers for internal exposure to acrylamide have been established, including urinary metabolites of acrylamide and its main metabolite glycidamide (GA), acrylamide mercapturic acid (AAMA) and glycidamide mercapturic acid (GAMA), and haemoglobin adducts (Hb-AA).

Here, the hazard assessment of acrylamide was performed by summarising available data from existing risk assessments. In the acrylamide risk assessment by the European Food Safety Authority (EFSA 2015), it was concluded that acrylamide potentially increases the risk of developing cancer in all age groups of the general population, since the main exposure to acrylamide happens via dietary intake. EFSA (2015) derived for acrylamide a Benchmark Dose Lower Confidence Limit ($BMDL_{10}$) of 0.17 mg/kg bw/d based on neoplastic effects in mice, and a $BMDL_{10}$ of 0.43 mg/kg bw/day based on peripheral neuropathy in rats.

Biomonitoring data from the HBM4EU aligned studies was used for cancer risk assessment of acrylamide, by utilising the urinary mass balance approach, previously used by Hays and Aylward (2008), to derive biomonitoring equivalent (BE) for acrylamide but using EFSA $BMDL_{10}$ as a starting point. Acrylamide intake was estimated based on the acrylamide urinary metabolite AAMA, using default bodyweight (30 kg for children, 70 kg for adults) and 24h urine excretion values (0.66 L for children, 1.7 L for adults) for the different population groups. Allometric scaling of $BMDL_{10}$ dose level of 0.17 mg/kg bw/d from mice to human was made using a default value of 4, which provided a point of departure (POD) for 10 % increase in tumour risk for humans at 42.5 µg/kg bw/d. This was calculated to correspond 2880 µg/L of urinary AAMA in a 70 kg adult (urinary volume of 1.7 L in 24 h). From this, a linear extrapolation to zero risk was performed. EFSA (2015) proposed also a $BMDL_{10}$ for non-neoplastic endpoints, 0.43 mg/kg bw/day based on peripheral neuropathy in rats. A health-based limit value of 0.0043 mg/kg bw/d was derived for peripheral neuropathy by using an assessment factor of 100 to account for uncertainties.

Geometric mean urinary AAMA concentrations in the HBM4EU aligned studies were in the range of 50–70 µg/L in children and 20-100 µg/L in adults. In previously published studies with general population, the corresponding range has been 30–73 µg/L. According to our cancer risk assessment, an acrylamide tumour risk of 1 : 1 000 at 0.425 µg/kg bw/d corresponding 28.8 µg/L of AAMA (in 70 kg adult) was estimated. This means that acrylamide cancer risk for children varied from 1:570 to 1:464 and in adults from 1:1384 to 1:288, when biomonitoring data from HBM4EU aligned studies were used as a starting point. These risks correspond to mean acrylamide intakes of 0.75–0.92 µg/kg bw/d in children and 0.31–1.47 µg/kg bw/d in adults, respectively. These levels are in line with the EFSA estimates on acrylamide intake via food (mean intake 0.4–1.9 µg/kg bw/d). Peripheral neuropathy risk was assessed by using the same urinary AAMA data from the aligned studies. RCRs were calculated for children and adults. In general, acrylamide levels were below the defined limit value 4.3 µg/kg bw/d. However, at 95th percentile of urinary AAMA levels,

there were two aligned studies performed with adults giving RCR values of 1.05 (INSEF-ExQAP, Portugal) and 1.75 (ESTEBAN, France), indicating increased risk for peripheral neuropathy.

This risk assessment contains several notable uncertainties. A clear threshold value for cancer risk cannot be established for genotoxic and mutagenic properties of acrylamide. Therefore, the cancer risk assessment is based on a linear extrapolation, which is likely to overestimate the risk at low exposure levels. In addition, cancer risk for humans was based on the most sensitive tumour type (Hardenian gland tumours) in mice, which also might result in overestimation of risk. Uncertainties of peripheral neuropathy risk assessment are related to species differences and assessment factor for deriving a health-based limit value. There is evidence of endogenous acrylamide formation (0.2-0.4 µg/kg bw/d) in humans, which also cause some uncertainty to Human Biomonitoring (HBM) based risk assessment. This is, however, estimated to represent lower uncertainty than uncertainties related to linear extrapolation of cancer risk levels from animal studies. In addition, individual variability of acrylamide metabolism can have influence on the HBM levels of acrylamide metabolites.

Monitoring of carcinogenic, mutagenic, and neurotoxic acrylamide is necessary to assess the exposure in the general population in EU. Consumption of roasted or baked starch-based foods and tobacco smoking have been associated with increased acrylamide exposure levels. The acrylamide exposure reduction lays mainly in personal choices regarding smoking and diet. In addition, efforts in non-smoking regulations and support for smoking cessation are important.

1.2 Introduction

Acrylamide is a low molecular weight, highly water soluble, organic compound produced for different uses in chemical industry. Enhanced public health concerns about exposure to acrylamide arose in 2002 when it was discovered that it is formed when certain foods are prepared at temperatures usually above 120 °C and low moisture. Acrylamide is detected in numerous baked or fried carbohydrate-rich foods, including frequently consumed foods in all population groups. It is also known to be formed in cigarette smoke. From experimental animal studies, acrylamide has been shown to have neurotoxic, carcinogenic, genotoxic and mutagenic effects (Muta. 1B, H340, Carc. 1B, H350 and STOT RE 1, H372** according to the CLP classification), possible/suspected immunotoxic and developmental toxic effects, and adverse effects on the reproductive function in males (Repr. 2, H361f*** CLP classification).

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 such as carcinogenicity and reproduction toxicity 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 (Carc. 1B; SVHC: substance of very high concern). Evidence from a limited number of epidemiological studies suggests that acrylamide may have some other adverse effects as foetal growth or immunotoxicity. A possible adverse effect of mixtures of acrylamide and other chemical compounds, particularly other carcinogens in food, should be taken into consideration for the risk assessment and needs to be further investigated. However, there are >25 epidemiological studies on acrylamide (alone) in the diet with an overall unclear outcome. Therefore, addressing the question of mixture effects is seen as problematic at this stage.

Humans are exposed to acrylamide through inhalation, ingestion and dermal uptake. The diet (including water) and cigarette smoke are the predominant acrylamide sources for the general population. For occupational exposure, inhalation and dermal contact at the workplace, where acrylamide is used or produced, are important routes of acrylamide exposure.

United States Environmental Protection Agency, USEPA (2010) has derived for acrylamide a chronic oral reference dose (RfD) 0.002 mg/kg/day for degenerative nerve changes by using human equivalent dose derived from a chronic rat study. In addition, an oral slope factor of 0.5 mg/kg/day was derived for carcinogenicity of acrylamide by summing the risks for different tumour types in rodents. However, age dependent adjustment factors (ADAF) should be used for age groups of <2 years (ADAF 10) and for 2 - 16 years (ADAF 3).

EFSA (2015) has published a full risk assessment of acrylamide in food, which concluded that acrylamide potentially increases the risk of developing cancer for consumers in all age groups. In addition to cancer, three possible critical endpoints were identified for acrylamide toxicity, i.e. neurotoxicity, effects on male reproduction and developmental toxicity. Benchmark Dose Lower Confidence Limit ($BMDL_{10}$) values of 0.43 mg/kg body weight per day (bw/d) were determined for peripheral neuropathy in rats and of 0.17 mg/kg bw/d for neoplastic effects in mice.

Health Canada has published an evaluation of Human Biomonitoring data in a health risk-based context: An updated analysis of population level data from the Canadian Health Measures Survey (Faure et al. 2020). These data were evaluated with the biomonitoring screening values, such as biomonitoring equivalents (BE). Additional cancer risks estimated from different concentration percentiles for the acrylamide exposure adduct biomarker in haemoglobin (GA-Val) in non-smokers were close to 10^{-3} (ranging from 5.12×10^{-4} at P5, flagged with high variability, to 2.25×10^{-3} at P95). Cancer risks in smokers were higher (ranging from 7.99×10^{-4} at P5 to 5.12×10^{-3} at P95).

Several policy-related questions were derived within HBM4EU for acrylamide. This risk assessment can answer partly to the questions 2, 4 and 5.

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 it possible to identify the best biomarkers of exposure to acrylamide that can be used in HBM studies?
7. Is there a possible mixture of effect between acrylamide and other chemical substances?
8. 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?

1.3 Methodology

The hazard assessment of acrylamide was performed by summarising available data from existing risk assessments. Biomonitoring data from HBM4EU aligned studies and aggregated data from WP10 was used for risk assessment of acrylamide by utilising the same urinary mass-balance approach previously used by Hays and Aylward (2008) for acrylamide but using EFSA (2015) dose-response analysis as a starting point.

Estimating the urinary AAMA concentration from the acrylamide uptake (or vice versa) is calculated with the equation as described below, which gives an estimate on urinary AAMA excretion at a defined cancer risk level extrapolated from the 2-year bioassay with mice and peripheral neuropathy risk extrapolated from rats.

Under steady-state exposure conditions consistent with chronic exposure, the daily elimination of AAMA on a molar basis should be equal to approximately 50 % of the intake. The urinary AAMA concentration (mg/L) from daily acrylamide intake (mg) can be estimated as follows:

$$\mathrm{AAMA} = \frac{1}{\mathrm{V24h}} \times \mathrm{AA} \times \mathrm{BW} \times \frac{\mathrm{MWAAMA}}{\mathrm{MWAA}} \times 0.5$$

AAMA is the amount of AAMA excreted in urine per litre (mg/L); V24h is 24h urinary volume (Table 1); AA is the total daily dose of acrylamide (mg); BW is the bodyweight (Table 1); MWAA and MWAAMA are the molecular weights of AA and AAMA (71.1 and 234.1 g/mol), respectively.

Table 1: Default body weights and 24 h urinary volumes used for different age groups

Age group	Bodyweight (kg)	24h Urinary volume (L)
Children <13 years	30	0.66
Adults >19 years	70	1.7

1.4 Hazard assessment

This assessment considers the neoplastic effects and peripheral neuropathy risk of acrylamide in general population which most likely are exposed via dietary intake. The data from human studies is inadequate for dose-response assessment for cancer endpoint (EFSA 2015). Therefore, EFSA (2015) selected as a reference point for neoplastic effects the value of 0.17 mg/kg bw/d derived as the lowest $BMDL_{10}$ from data on incidences of Harderian gland adenomas and adenocarcinomas in male B6C3F1 mice exposed to acrylamide for two years in the National Toxicology Program (NTP) study. However, it was noted that the Harderian gland is an organ largely absent in adult humans, but that in rodents this organ is a sensitive target tissue to detect compounds that are both genotoxic and carcinogenic. The Hardenian gland was considered the most sensitive target tissue in rodent bioassays, and a conservative endpoint for acrylamide risk assessment of neoplastic effects in humans. It was inappropriate to establish a tolerable daily intake (TDI) for acrylamide since acrylamide and its metabolite glycidamide are genotoxic.

EFSA (2015) proposed also $BMDL_{10}$ for non-neoplastic endpoints – peripheral neuropathy in rats as 0.43 mg/kg bw/day for the most relevant and sensitive endpoint for neurotoxicity, i.e. the incidence of peripheral nerve (sciatic) axonal degeneration observed in F344 rats exposed to acrylamide in drinking water for two years in the NTP study.

1.5 Exposure assessment using Human Biomonitoring data

In EFSA (2015) risk assessment it was concluded that acrylamide potentially increases the risk of developing cancer for consumers in all age groups. Acrylamide was found at the highest levels in solid coffee substitutes and coffee, and in fried potato products. Acrylamide is a component of tobacco smoke, and hence smoking as well as passive smoking are an important source of human exposure to acrylamide.

Mean and 95th percentile dietary exposures across surveys and age groups were estimated at 0.4 to 1.9 µg/kg bw/d and 0.6 to 3.4 µg/kg bw/d, respectively.

The most vulnerable groups for the possible adverse effect of acrylamide exposure are infants, toddlers, children and pregnant women. Workers at the industrial site and manufacturing have been shown to be highly exposed. Earlier Human Biomonitoring (HBM) data on acrylamide in population representative studies in Europe have been limited (Table 3). Acrylamide was, however, included in HBM4EU aligned studies to get a more comprehensive picture on acrylamide exposure. HBM urinary AAMA concentrations measured in HBM4EU aligned studies are presented for children in Table 4 and for adults in Table 5.

Three main types of biomarkers have been identified for internal exposure to acrylamide including urinary metabolites and adducts with haemoglobin. Acrylamide either reacts with glutathione, and the resulting conjugate is further metabolised to N-acetyl-S-(2-carbamoylethyl)cysteine or better known as acrylamide mercapturic acid (AAMA). The metabolism of acrylamide by hepatic CYP2E1 leads to the formation of glycidamide (GA). The detoxification by glutathione conjugation leads to the excretion of N-Acetyl-S-2-(2-hydroxy-2-carbamoylethyl)cysteine also known as glycidamide mercapturic acid (GAMA). The mercapturic acids are excreted in the urine. Both acrylamide and GA form adducts with thiol and amine groups in haemoglobin and other proteins as well as in DNA. Data gained from urinary metabolite determinations reflect exposure to acrylamide over recent two days. In contrast, the haemoglobin adducts reflect the medium-term exposure over about 120 days.

Table 3: Urinary AAMA concentrations measured in different HBM studies

Study region (sampling year)	Age group	Number of study participants (n)	AAMA (µg/L)	Reference
Germany (2002)	adults	60	P50: 41.6	Urban et al. 2006
Germany (<2005)	16-67	29	P50: 60	Boettcher et al. 2005
Germany (>2005)	5-6	110	P50: 36 P95: 152.7	Heudorf et al. 2009
South Korea (2009)	18-69	1874	P50: 29.42 P95: 157.91	Lee et al. 2014; 2019
South Korea (2011)	10-13	31	P50: 68.1	Ji et al. 2013
Poland (2012)	20-40	93	P50: 20.9	Mojska et al. 2016
Germany (2015-2017)	3-17	2260	P50: 71.8 P95: 267	Schwedler et al. 2021
Spain (2015)	20-45	114	P50: 73 P95: 266	Fernandes et al. 2022

AAMA = acrylamide mercapturic acid, P50 = 50th percentile, P95 = 95th percentile

Table 4: Urinary AAMA concentrations measured in HBM4EU acrylamide aligned studies for children

HBM4EU acrylamide aligned study (Children)	Urine sample type	AAMA (µg/L) GM	AAMA (µg/L) GM CI	AAMA (µg/L) P95
Italy, EPIUD_NAC II n = 300	Spot sample	66.26	60.31 – 72.80	208.88
Norway, NIPH_NEB II n = 299	Spot sample	55.76	51.53 – 60.35	189.07
Germany, UBA_GerES V n = 300	First morning sample	68.54	63.63 – 73.83	179.15
France, ANSP_ESTEBAN n = 300	First morning sample	67.69	62.62 – 73.16	207.28

AAMA = acrylamide mercapturic acid, GM = geometric mean, CI GM = approximate 95% confidence interval for the GM, N = number of study participants, P95 = 95th percentile

Table 5: Urinary AAMA concentrations measured in HBM4EU acrylamide aligned studies for adults

HBM4EU acrylamide aligned study (Adults)	Urine sample type	AAMA (µg/L) GM	AAMA (µg/L) GM CI	AAMA (µg/L) P95
Germany, UBA_ESB n = 180	24h sample	20.81	17.66 – 24.52	66.77
Luxemburg, LNS_Oriscav-Lux2 n = 204	Spot sample	56.50	49.69 – 64.24	232.48
Iceland, UI_DIET_HBM n = 203	Spot sample	57.74	50.74 – 65.71	245.93
Portugal, INSA_INSEF-ExQAP n = 294	First morning sample	85.57	77.96 – 93.92	336.53
France, ANSP_ESTEBAN n = 300	First morning sample	99.95	90.18 – 110.79	510.83

AAMA = acrylamide mercapturic acid, GM = geometric mean, CI GM = approximate 95% confidence interval for the GM, N = number of study participants, P95 = 95th percentile

1.6 Risk characterisation and uncertainty analysis

In EFSA (2015) risk assessment it was concluded that acrylamide potentially increases the risk of developing cancer for general population in all age groups since the main exposure to acrylamide happens via dietary intake. EFSA (2015) derived for acrylamide a neoplastic reference point $BMDL_{10}$ of 0.17 mg/kg bw/d for mice. Allometric scaling of this dose level of 0.17 mg/kg bw/d from mice to human using a default value of 4 provides a point of departure (POD) for 10 % increase in tumour risk for humans of 42.5 µg/kg bw/d.

Using this value, the following risk numbers for a 70 kg human adult were linearly extrapolated and calculated by utilising the described urinary mass-balance method:

A tumour risk of 1 : 10 at the $BMDL_{10}$ of 42.5 µg/kg bw/d corresponding 2880 µg/L of urinary AAMA (in a 70 kg adult and 1.7 L urinary volume in 24 h)

A tumour risk of 1 : 1 000 at 0.425 µg/kg bw/d corresponding 28.8 µg/L of AAMA

A tumour risk of 1 : 10^5 at 0.00425 µg/kg bw/d corresponding 0.288 µg/L of AAMA

A tumour risk of 1 : 10^6 at 0.000425 µg/kg bw/d corresponding 0.0288 µg/L of AAMA

Acrylamide cancer risk assessment by utilising biomonitoring data for adults and children can be found in Table 6. The estimated acrylamide cancer risks for different population groups in HBM4EU aligned studies are presented in Table 7.

Table 6: Linearly extrapolated acrylamide cancer risks and their corresponding urine AAMA levels for different age groups by using urinary mass-balance method

Acrylamide tumour risk	AAMA, Children (µg/L)	AAMA, Adults (µg/L)
1:10 at 42.5 µg/kg bw/d	3180	2880
1:1000 at 0.425 µg/kg bw/d	31.8	28.8
1:105 at 0.00425 µg/kg bw/d	0.318	0.288
1:106 at 0.000425 µg/kg bw/d	0.0318	0.0288

AAMA = acrylamide mercapturic acid

Table 7: Estimated acrylamide cancer risk for different population groups of HBM4EU aligned studies

Population group	AAMA (µg/L)	Acrylamide (µg/kg bw/d)	Tumour risk
Children	GM: 55.76 – 68.54 P95: 179.15 – 208.88	GM: 0.75 – 0.92 P95: 2.39 – 2.79	1:570 to 1:464 1:178 to 1:152
Adults	GM: 20.81 – 99.95 P95: 66.77 – 510.83	GM: 0.31 – 1.47 P95: 0.99 – 7.54	1:1384 to 1:288 1:431 to 1:56

AAMA = acrylamide mercapturic acid, GM = geometric mean, P95 = 95th percentile

EFSA (2015) proposed also $BMDL_{10}$ for non-neoplastic endpoints – peripheral neuropathy in rats as 0.43 mg/kg bw/day. If assessment factor of 100 is used to account for uncertainties a health-based limit value of 0.0043 mg/kg bw/d is derived for peripheral neuropathy.

Using this value of 4.3 µg/kg bw/d, the following corresponding AAMA concentrations for a 30 kg child and 70 kg adult were calculated by utilising the urinary mass-balance method using the same FU_E as earlier:

Daily acrylamide exposure of 4.3 µg/kg bw/d corresponds urinary AAMA in

- 30 kg children: 321.7 µg/L
- 70 kg adults: 291.4 µg/L

Risk characterisation ratios (RCR) were calculated for peripheral neuropathy in HBM4EU aligned study urinary AAMA concentrations at geometrical mean and 95th percentile levels in children and adults (Table 8).

Table 8: Estimated acrylamide peripheral neuropathy risk for different population groups of HBM4EU aligned studies

Population group	AAMA (µg/L)	Acrylamide (µg/kg bw/d)	RCR for peripheral neuropathy
Children	GM: 55.76 – 68.54 P95: 179.15 – 208.88	GM: 0.75 – 0.92 P95: 2.39 – 2.79	0.17 – 0.21 0.56 – 0.65
Adults	GM: 20.81 – 99.95 P95: 66.77 – 510.83	GM: 0.31 – 1.47 P95: 0.99 – 7.54	0.07 – 0.34 0.23 – 1.75

AAMA = acrylamide mercapturic acid, GM = geometric mean, P95 = 95th percentile, RCR = risk characterisation ratio

1.6.1 Uncertainty analysis

Uncertainties affecting this acrylamide cancer risk assessment have been gathered to Table 9.

Genotoxic and mutagenic properties of acrylamide cause uncertainty to cancer risk assessment since a threshold value for cancer risk cannot be established. In this risk assessment, a linear extrapolation of cancer risk for humans was performed from a $BMDL_{10}$ reference value derived for neoplastic effects observed in mice. An allometric uncertainty scaling value of 4 was used for interspecies differences between mouse and human. Linear extrapolation is considered as a conservative approach resulting rather to the overestimation of the risks at low levels than to the underestimation. Overestimation of the risks is supported by the lack of epidemiological evidence on the carcinogenicity of acrylamide in humans. Also, the starting point for the dose-response analysis was the most sensitive tumour type in mice, which was Harderian gland tumours. These tumours have no counterpart in humans and are sensitive indicators of carcinogenicity in rodents. Thus, it might be that humans are less sensitive to the carcinogenicity of acrylamide than rodents and the use of allometric scaling factor for interspecies extrapolation may have been unnecessary.

Uncertainties related to peripheral neuropathy risk assessment are related to species differences and assessment factor for deriving a health-based limit value. Assessment factor of 100 was used to extrapolate the $BMDL_{10}$ value of 0.43 mg/kg bw/d from rat to human.

There is evidence of endogenous acrylamide formation in humans which can cause uncertainty to HMB and risk assessment. Endogenous acrylamide levels have been determined to be 0.2-0.4 µg/kg bw/d (Ruenz et al. 2015, Goempel et al. 2017). In addition, individual variability of acrylamide metabolism can have influence on HBM levels of acrylamide metabolites. Interindividual variation ranging from 20 % to 30 % have been identified in humans for urinary excretion of AAMA and GAMA. This can be considered to have, however, a much smaller impact on the risk assessment than uncertainties in dose-response analysis. It should be also noted that acrylamide external exposure estimates based on HBM data are not so different from the external exposure estimated made by EFSA on the basis of food consumption data (Mean 0.4-1.9 and P95 of 0.6-3.4 µg/kg bw/d versus 0.3-1.5 and P95 1.0-7.5 µg/kg bw/d in adults).

Tobacco smoking can cause uncertainty to risk assessment since smoking elevates acrylamide levels which have been identified as elevated levels of AAMA and GAMA metabolites in urine of smokers. For example, in German Environmental Survey 2014–2017 (GerES V) HBM study with children and adolescents smokers had about 2.5-fold higher AAMA levels than non-smoking participants (Schwedler et al. 2021).

Table 9: Uncertainties affecting acrylamide cancer and neuropathy risk assessment

Uncertainty factor	Impact on acrylamide risk assessment
No threshold (genotoxic), linear extrapolation	↑↑↑
POD from mice and rat data	↑↑
Endogenous acrylamide	↑
Individual metabolism	↑↓
Spot urine sample First morning sample	↑↓
Urinary mass balance -method used for reverse calculation	↑↓

↑ overestimation; ↓ underestimation; ↑↓ overestimation and underestimation; POD = point of departure

One source of uncertainty is acrylamide urinary biomarkers because urinary metabolite determinations reflect exposure to acrylamide over recent two days and acrylamide haemoglobin adducts represent a more chronic exposure. Moreover, because majority of the HBM urine samples were spot samples and first morning samples, concentrations of acrylamide biomarkers can vary substantially.

1.7 Discussion and conclusions

General discussion and conclusions on the work performed

Geometric mean of acrylamide urinary metabolite AAMA concentrations in HBM4EU aligned studies were in the range of 50-70 µg/L in children and 20-100 µg/L in adults. In previously published studies with general population the corresponding range has been 30-73 µg/L. These biomarker levels correspond to mean acrylamide intakes of 0.75 – 0.92 µg/kg bw/d in children and 0.31 – 1.47 µg/kg bw/d in adults, respectively.

According to our assessment, an acrylamide tumour risk level of 1 : 1 000 at 0.425 µg/kg bw/d corresponds to 31.8 and 28.8 µg/L of AAMA (in children and adults, respectively). This means that acrylamide cancer risk in the population varies 1:1384 to 1:288 when calculated using mean (GM) biomarker levels from HBM4EU aligned studies. The differences between adults and children exposure to acrylamide in HBM4EU aligned studies were minor. In addition, the calculated tumour risk for children was in the range of corresponding risk for adults. However, it should be noted that the tumour risk has been calculated for lifetime and children will eventually become adults.

EFSA (2015) estimated mean and 95th percentile dietary exposures of acrylamide across surveys and age groups were at 0.4 to 1.9 µg/kg bw/d and 0.6 to 3.4 µg/kg bw/d, respectively. In this study, based on biomarker data from HBM4EU aligned studies, acrylamide mean and 95th percentile intakes were estimated to be at similar levels, 0.3-1.47 to 0.99-7.54 µg/kg bw/d. In the EFSA (2015) acrylamide risk assessment, the margin of exposure (MOE) for the cancer related effects of acrylamide ranged from 425 for average adult consumers down to 50 for high consuming toddlers, which is in the same order of magnitude as observed in our assessment. According to EFSA, substances that are genotoxic and carcinogenic an MOE of 10 000 or higher is of low concern for public health.

This acrylamide cancer risk assessment is conservative since the risk was based on the linear extrapolation from the mice carcinogenicity data using $BMDL_{10}$ value derived from the most

sensitive tumour type as a POD. In contrast, results from human studies provide limited and inconsistent evidence of increased cancer risk in association with dietary exposure to acrylamide.

Peripheral neuropathy risk was assessed by using the same urinary AAMA data from aligned studies. RCRs were calculated for children and adults which indicated risks under the defined 4.3 µg/kg bw/d. However, at 95th percentile urinary AAMA levels there were two aligned studies performed with adults giving RCR values of 1.05 (INSEF-ExQAP, Portugal) and 1.75 (ESTEBAN, France) indicating increased risk for peripheral neuropathy. According to EFSA (2015) estimates across surveys and age groups, MOE values for the neurotoxic effects ranged from 1 075 to 226 for the mean exposure, and from 717 to 126 for the 95th percentile exposure.

Results in the light of policy questions

PQ: 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?

The exposure levels of acrylamide in the HBM4EU aligned studies are similar in the general population as previously reported by EFSA and by published HBM studies. Concerning cancer risk, our risk assessment suggested a clear concern for general population. This is in accordance with the earlier risk assessment by EFSA. Regarding peripheral neuropathy risk, in general acrylamide levels were below the provisional HBM-GV derived. However, at 95th percentile of urinary AAMA levels, there were two aligned studies performed with adults giving RCR values of 1.05 (INSEF-ExQAP, Portugal) and 1.75 (ESTEBAN, France), indicating a concern for peripheral neuropathy. This risk assessment contains several potential uncertainties, which are mainly related to the extrapolation of the risks for humans by using animal data and may lead to overestimation of risk. The association of acrylamide to foetal growth, or the dependence of health risk on long term or intermittent exposure to acrylamide were not considered here.

PQ: Are the health risks dependent on age and gender? Which population groups are more at risk?

Based on this work, it is not possible to identify age or gender dependencies.

Recommendations for the regulatory risk assessment

Continuation of the monitoring of acrylamide is necessary to assess the exposure in the EU general population (EC 2017/2158). Consumption of roasted or baked starch-based foods and tobacco smoking have been associated with increased acrylamide exposure levels. The acrylamide exposure reduction lays mainly in personal choices regarding smoking and diet. In addition, efforts in non-smoking regulations and support for smoking cessation are important.

Future prospects

The continuous monitoring of acrylamide exposure levels in general population is important in the future and should include HBM data from several other European countries. In addition, making general population more aware with information campaign of acrylamide and its sources could reduce the levels.

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2 Arsenic full RA report

Risk assessment for arsenic

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2.1 Summary

Recent work related to background levels of inorganic arsenic (iAs) in food and environment in the European Union (EU) is summarised; most data included are from studies from 2000 until 2020. Exposure of different population groups is calculated by combining intake and iAs concentrations of food items to obtain daily doses. Conservative approach was used for iAs bioavailability - taken as 100%. Additionally, Human Biomonitoring (HBM) studies are gathered and iAs daily doses are calculated from urine iAs metabolites by reverse dose calculation. Both daily iAs dose estimates were used for the assessment of cancer risks – based on dose-response relationship proposed by JEFCA (2011) and ECHA (2013). Uncertainties and limitations of the risk assessment were discussed along with the relevance of the findings in the context of the incidence of selected endpoints (i.e., lung cancer) in the entire general population in the EU. Throughout the text food intake is used as synonym for food and drinking water intake.

Current exposure of the average EU population to arsenic from drinking water is low due to the limited presence of iAs in drinking water: GM 0.19 µg L^{-1}, P75 0.47 µg L^{-1}, 99 % of samples below the maximum permissible value of 10 µg L^{-1} in EU (Banks et al. 2015). The main source of iAs is diet, especially rice and other cereals and seafood.

Average exposure levels (daily doses) calculated from food intake and iAs concentrations in food in our study are in the range of 0.07 to 0.20 µg kg^{-1} bw/day for seven age-stratified population groups (from toddlers to very elderly) and P95 levels lie between 0.19 and 0.64 µg kg^{-1} bw/day.

In scientific literature and within this project, we identified 28 sets of HBM data on arsenic speciation in the urine of different population groups in the EU including from 11 to 1737 participants exposed to “normal” levels of iAs. An estimated daily dose of iAs from these urinary levels (including iAs, MMA and DMA) was re-calculated according to Hays et al. (2010), who assumed the linear relationship between the steady-state concentration of arsenic in urine and a daily dose of iAs. On average, estimated daily dose was 0.16 ± 0.07 µg kg^{-1} bw/day (average of geometric means) and average of P95 was 0.48 ± 0.19 µg kg^{-1} bw/day, both agreeing well with daily dose calculated from food intake for children, adolescents and adults.

Simple multiplication of estimated daily dose of iAs from HBM studies (0.16 µg kg^{-1} bw/day) with a proposed lifetime excess lung cancer risk of 1.7 x 10^{-3} per 1 µg kg^{-1} bw/day gives a number of 2.7 x 10^{-4} (multiplication of standard deviation is not done since it does not represent the actual dispersion or uncertainty of the values used). Credible justification of assumptions, necessary to assure the credibility of such calculation is beyond the scope of this work, therefore, the calculated value must be interpreted with caution. Excess lifetime cancer risk factor (dose-response function) is used to estimate the number of additional cancer risk in the exposed population due to iAs exposure. Since the risk assessment is based on the assumption of the linear relationship between the exposure and cancer risk, this approach can be considered as conservative, overestimating the risk especially at low exposure levels. This needs to be considered when using these results.

In addition to the uncertainties related to the dose-response of arsenic caused cancer especially at low exposure levels there are additional uncertainties, specific for the use of HBM data. These include:

- uncertainty related to the bioavailability of iAs from different food items,
- representativeness of populations and applicability of epidemiological data
- overestimation of iAs exposure due to the wide spread presence of DMA in food,
- analytical challenges related to speciation of As species
- inter-individual differences, including individual susceptibility factors

2.2 Introduction

Arsenic is present in the environment in a form of numerous compounds, which differ greatly in their properties. Due to the different toxicity, knowledge of the type of compound (speciation) is imperative, since total arsenic concentrations say nothing about the potential health risk (Luvonga et al. 2020).

Toxicologically most relevant arsenic compounds (Luvonga et al. 2020, Sattar et al. 2016)

Inorganic arsenic (iAs) in a reduced (arsenite) and oxidised form (arsenate) are most toxic. Arsenite is more toxic and carcinogenic. Arsenite targets sulfhydryl group-containing enzymes and disturbs several metabolic pathways. Arsenate replaces the phosphate group and is harmful in ATP production. Both of them can be found in soil, air, water and in some food.

Monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA), both of which contain arsenic in the pentavalent form, are the product of iAs methylation in the environment and organisms including humans. Their toxicity is about 20 times lower than toxicity of As(III) and As(V). Most DMA can be found in seafood, rice, and some mushrooms. MMA(III) and DMA(III), in which arsenic is in trivalent form, are much more toxic but much less frequently as other arsenic compounds present in the environment and even then at much lower concentrations (Luvonga et al. 2020).

Non-toxic arsenic compounds

Arsenobetaine is very common in the marine environment and can be found in high concentrations in seafood. After ingestion, it is excreted unchanged in urine and is considered non-toxic. Tetramethylarsonium ion, present in seafood in very low concentrations, also seems to exhibit no toxicity (Sattar et al. 2016).

Compounds with unknown toxicity and/or low abundance in the environment and food

Arsenosugars are present mainly in seaweed and molluscs, possibly at fairly high concentrations. In humans, they are degraded to several products with unknown toxicity and to DMA (Francesconi et al. 2002). Arsenolipids are present in the fatty tissue of marine organisms (Al Amin et al. 2020). Their toxicity is largely unknown and the major metabolite after ingestion of cod liver containing arsenolipids is DMA (Schmeisser et al. 2006). DMA formed from ingested arsenosugars and arsenolipids and excreted in urine is a confounder making realistic estimation of exposure to iAs difficult (iAs exposure is evaluated by its presence and presence of its metabolites MMA and DMA in urine). Trimethylarsine oxide and arsenocholine are much less toxic than MMA and DMA (Sattar et al. 2016) and their concentrations in food are almost always negligible. Organoarsenic compounds in which oxygen in the abovementioned compounds is replaced by sulphur - the so-called thiolated arsenic compounds are usually less abundant in food. Their toxicity varies from compound to compound and is in many cases unknown (Herath et al. 2018, Luvonga et al. 2020).

2.2.1 Arsenic compounds in the environment

The soil in the European Union (EU) generally contains low levels of arsenic with exception of some hot - spots, mainly connected to natural origin, mining and industrial activities (Tarvainen et al. 2013). Over 95 % of topsoils in Europe contain less than 20 mg kg^{-1} of arsenic (P75 < 10 mg kg^{-1}), including high proportion below 3 mg kg^{-1} (P25). Somewhat higher concentrations are most often found in mountains (the Alps, Carpathians, Masif Central of France, and the Pyrenees) (Tóth et al. 2016). The Working group on arsenic, cadmium, and nickel compounds (European commission 2001) evaluated the daily intake of arsenic from soil and dust to be 0.0017 µg kg^{-1} bw/day at an average arsenic concentration of 5 mg kg^{-1} in soil.

The level of arsenic in natural waters generally ranges between 1 and 2 µg L^{-1} (US NRC 1999); all arsenic in drinking water is in inorganic form. The guideline value for drinking water is 10 µg L^{-1}, although the concentration associated with an excess life-time skin cancer risk of 10^{-5} was calculated to be 0.17 µg L^{-1} (WHO 2011, US EPA 2001). For practical reasons, 10 µg L^{-1} is accepted as maximum. A survey of 579 tap water samples from EU and non-EU European countries showed that median arsenic concentration in tap water is 0.19 µg L^{-1} (P75 0.47 µg L^{-1}, Banks et al. 2015) with 99 % of samples below the maximum permissible value of 10 µg L^{-1} in EU. Dieter et al. (1994) found that 93.5% of drinking water samples in Germany were below 2 µg L^{-1} of arsenic, out of that 74.4% were below the detection limit and 1.2 % above 10 µg L^{-1}. In some areas in European countries drinking water contains arsenic levels above current provisional guideline value of 10 µg L^{-1}. In some areas of Romania, Hungary, Slovakia, Greece, Ireland, Croatia, Serbia, Denmark, Finland and Italy elevated arsenic in drinking water was reported (Van Halem et al. 2009).

According to data for 2017, in most parts of the EU, the mean annual level of arsenic in the air is low, mostly below the detection limit (< 1 ng m^{-3}) or 1 - 3 ng m^{-3} (Annual mean As, 2017). Working group on arsenic, cadmium, and nickel compounds (European Commission 2001) evaluated the daily intake of arsenic from the air at average 2 ng m^{-3} arsenic to be 0.0003 µg kg^{-1} bw/day.

2.2.2 Inorganic As concentrations in diet (food and drinking water) in EU

A comprehensive document on arsenic in food in the EU was published by European Food Safety Authority (EFSA 2009, 2014) and supplemented in 2021 with one, focused on iAs concentrations and exposure via food (EFSA 2021). Table 1 gives some arsenic and iAs concentrations in a broad range of food items from European markets reported in recent studies (2015 - 2021). We narrowed the data taking into account only studies in which a high number of products from many food groups were analysed with analytical procedures with low detection limits. Among the majority of foods with normally low levels of arsenic, some food groups need special attention:

The highest total arsenic concentrations of all foods are found in marine fish, molluscs, and crustaceans. Inorganic As content in seafood is in general low but should be taken into account when seafood represents a considerable proportion of the diet. Edible seaweeds can contain high concentrations of iAs and arsenosugars (Banach et al. 2020, EFSA 2021, Luvonga et al. 2020). Potentially elevated content of DMA, arsenosugars, and arsenolipids in seafood is relevant for the estimation of dietary intake of iAs via urine analysis because all of these compounds yield increased DMA content in urine (Al Amin et al. 2020). Anyway, a lack of data on arsenic toxicity in humans and other mammals consuming a significant amount of seafood arsenic (mainly arsenobetaine, arsenolipids and arsenosugars) provide supporting evidence against arsenic acute toxicity due to the exposure from seafood consumption compared to exposure to elevated iAs from drinking water (Luvonga et al. 2020; Borak et al. 2007).

Cereals are an important source of arsenic consumed with food. The highest arsenic content of all cereals is normally found in rice, which on European market contains on average about 90 µg kg^{-1} of iAs and 20 - 30 µg kg^{-1} DMA (both dry weight, Šlejkovec et al. 2020). The EU specifies maximum allowable iAs levels of 200 µg kg^{-1} (white rice) and 250 µg kg^{-1} (parboiled and brown rice); rice products intended for infants and small children are allowed to contain less iAs (100 µg kg^{-1}). Additionally, regular consumption of rice waffles by children is discouraged (BFR, 2014). This is supported by the Hoge Gezondheidsraad Belgium (2018).

Other cereals including wheat can also contain more arsenic than non-cereal plants (Upadhyay et al. 2019). Further attention should thus be given to wheat, and potentially other cereals as well.

Table 1: Recent data for total and inorganic As (iAs) concentrations in foods from European markets from studies with low detection limits and a large number of samples (µg kg^{-1})

	As_{total}/iAs, AGES 2015	As_{total}/iAs González et al. 2019	iAs, Cubadda et al. 2016	iAs EFSA 2014	iAs, EFSA 2021
Location, No. of samples	**Austria, 1080, mean values**	**Spain, grouped as 20 of each samples**	**Italy, > 3000, mean values**	**> 100000, estimated as 70% of As_{total}**	**5985 food and 7623 water samples (mean)**
Detection limit		**2 µg/kg**	**1 – 3 µg/kg**	**10 – 70 µg/kg for most data**	**0.001-35 µg/kg**
Vegetables	16 – 26/ 9 - 19	1/1	0.1 – 8.5 2.5 potatoes	0 – 57	0 - 20
Fruit		2/2	0.3 – 4.4	0 – 9	0 - 20
Meat and meat products	0 – 18/ 0 - 12	3/3	0.4 – 1.4	0 – 16	0 - 10
Cereals, bread, pasta, flour	0 – 20/ 0 - 14	47/34	1.7 – 4.2	0 – 32	0 - 23
Cereals, grains without rice		47/34	22 - 36	0 – 49	9 - 18
Rice	145/100		13.7	92 – 110	66 - 148
Milk, milk products	0 – 1/0 - 1	1/1	0.3 – 0.9	0 – 27	0.07 - 4
Eggs		<2/<2	0.3	0 – 9	0 - 10
Fish	1370-3200/31-50	3590/<2	2.5	0 – 49	4 – 26.4
Crustaceans, bivalves			28.3	0 – 131	15 – 26.7 1.3 - 110
Drinks (coffee, thee, beer, vine)	0 – 20/ 0 - 14		0.9 – 2.7	0 – 14	0.3 - 6
Fruit juices	0 – 15/0 - 11		1.7	0 – 11	1 - 17
Drinking water			0.6 – 2.1	0 – 2	0.6 – 2.9
Mushrooms				0 – 220	13 - 45
Seaweed	2700/1900			65 – 450	63 - 9134
Oils, fats		<2/<2	0.3 – 0.8	0 – 45	

2.3 Methodology

2.3.1 Hazard assessment and dose-response

A major concern in the case of inorganic arsenic is its ability to cause cancer. There are existing evaluations on the cancer risk caused by inorganic arsenic. Available dose-responses are based on the default non-threshold approach, since it has been generally considered that the scientific data is not sufficient to set a threshold for the carcinogenic effects of inorganic arsenic. However, there are also reports trying to set a threshold below which there are no expected cancer risks due to iAs exposure from drinking water (Tsuji et al. 2019). It should be noted that also this approach includes uncertainties. In this risk assessment we have used non-threshold approach based on earlier evaluations by the regulatory bodies.

The excess lifetime risk of developing lung cancer from the intake of 1 µg kg^{-1} bw/day, is 1.7 per 1000 based on the no-threshold approach (JECFA 2011, ECHA 2013). The newest US FDA (2016) report estimates that 383 bladder and 1123 lung cancers per million are expected for 1 µg kg^{-1} bw/day (together 1.506 per 1000). Cancer risk was calculated using both cancer slopes, i.e. $1.7 \cdot 10^{-3}$ per µg kg^{-1} bw/day for only lung cancer (JECFA 2011, ECHA 2013) and $1.506 \cdot 10^{-3}$ per µg kg^{-1} bw/day for lung and bladder cancer (US FDA, 2016). ECHA recognised the possibility of a threshold cancer effect, but mentions that available data did not allow to derive a threshold. Therefore, the default non-threshold approach was used. Two studies supporting the proposed excess lifetime cancer risk slope factor were done in north-eastern Taiwan and only included individuals of 40 age and older (Chen 2010a, 2010b) and a follow-up period of only 11.5 years.

Despite the proposed use of the excess lifetime cancer risk slope estimate ECHA (2013) warns that "it is probable not wise to conduct linear extrapolations beyond the range of exposures experienced by the Taiwanese drinking water cohort, which ranged from about 2 to 25 µg As kg^{-1} bw/day as the shape of the response curve is uncertain." Linear extrapolation is considered (ECHA 2013) as the most appropriate default position, despite the recognition that there is also some mechanistic evidence supporting a threshold for the carcinogenicity (Sidhu et al 2015, Cohen et al. 2019, Tsuji, 2021, Lamm et al 2021, Kobets et al 2019, Thamalingan et al 2019).

2.3.2 Exposure assessment

We estimated the exposure to iAs in two ways:

a) a) through the calculation of a daily iAs dose (oral route of exposure), based on the iAs concentration in selected food items and their intakes under supposition of 100% bioavailability with known overestimation as not all food items are completely digested and absorbed; and

b) b) by re-calculating the daily iAs exposure from available HBM data according to Hays et al. (2010) (reverse dosimetry).

2.3.2.1 Exposure assessment based on food intake calculations

iAs exposure was calculated by multiplying the mean and P95 consumption of each food for each country (FoodEx 2016) with the corresponding iAs concentration:

$$Daily\ dose\ of\ iAs\ = \frac{\sum(iAs\ concentration_i\ \times\ food\ intake_i)}{body\ weight}$$

Minimum, mean, and maximum iAs concentration in food items from recent studies with a high number of samples and low detection limits were used (Cubadda et al. 2016, Chekri et al. 2019, Jackson et al. 2012). Calculated values are averages of all included countries. For the calculation, seven age-stratified population groups from various countries were included:

- Infants (5 countries): up to 12 months, for the calculation 1-year-old, 10 kg, (https://stats.areppim. com/stats/statsweightboysch.htm),
- Toddlers (6 countries): from 12 to 36 months of age, for the calculation 3 years old, 14 kg https://www.who.int/tools/child-growth-standards/standards/weight-for-age)
- Other children (14 countries): 3 – 10 years old, for the calculation 8 years old, 30 kg
- Adolescents (14 countries): 10 – 18 years old, for the calculation 14 years old, 50 kg, 14 countries
- Adults (16 countries): 18 - 65 years of age, 70 kg
- Elderly (13 countries): 65 - 75 years of age, 70 kg
- Very elderly (9 countries): from 75 years of age and older, 70 kg

2.3.2.2 Exposure assessment based on HBM data

From the published literature and from the HBM4EU repository we gathered data of urinary excretion of iAs+MMA+DMA (metabolites representing iAs exposure) for occupationally unexposed and exposed European population groups between years 2010 – 2020. For unexposed populations, 28 sets of data from Europe were identified, all reporting speciated As results in urine.

We used these data to calculate exposure in a form of iAs daily dose according to Hays et al. (2010), who assumed a linear relationship between the steady-state concentration of arsenic in

urine (C_V in µg L^{-1} on a volume basis or C_C, in µg $g^{-1}_{creatinine}$ on creatinine-adjusted basis) and a daily dose of iAs (D_A) given by the following two relationships:

$$C_V\ (\mu g/L) = 24.2 \frac{\mu g/L}{\mu g/kg/day} * D_A\ (\mu g/kg/day)$$

$$C_C\ (\mu g/g_{creatinine}) = 31.1 \frac{\mu g/g_{creatinine}}{\mu g/kg/day} * D_A\ (\mu g/kg/day)$$

Daily iAs doses calculated from urinary As excretion were compared with calculated iAs exposure estimates based on food consumption and were also used for the calculation of cancer risk.

2.3.3 Susceptibility factors

People vary considerably in their ability to metabolise arsenic, which is reflected by the widely varying proportions of iAs and its methylated forms in urine and blood. iAs is enzymatically biotransformed by arsenic methyl transferase (AS3MT) to monomethyl-As and further to dimethyl-As; both metabolites have higher urine elimination rates than iAs. Variability depends on (NRC, 2013):

- Life stages.
- Sex differences in the metabolism of iAs are well known, however studies with arsenic-related outcomes are very rarely evaluated by sex.
- Genetics of As metabolism and toxicity - polymorphic forms of the AS3MT enzyme system, either alone or in combination with polymorphic forms of some other enzymes may play role in differences and susceptibility to the various clinical manifestations of As effects. Wide variations in the relative abundance of genetic variants of the AS3MT system have been reported between populations and among individuals pointing on adaption mechanisms. Genetic factors my confer susceptibility or resistance to its exposure.
- Nutritional deficiencies (of folate, selenium, proteins) causing reduced methylation capacity of iAs. For instance, the status of selenium is significantly lower in EU populations than in the USA population, thus, the results of epidemiological studies could be site-specific and hardly comparable without adjustment for selenium which is well-known arsenic detoxifiers (Zeng et al, 2005; Falnoga et al 2014). It is also important to know that much of South Asia have a high folate deficiency, while mandatory folic acid fortification programs have been introduced in several countries (including the USA, Canada, Costa Rica, Chile, and South Africa) but not in Europe.
- Pre-existing disease (diabetes, cardiovascular disease, renal disfunction, specific physiological conditions (pregnancy), smoking, alcohol consumption, all affecting As metabolism.
- Synchronic exposures to multiple substances (mixtures).

All these factors in combination are rarely implemented in HBM studies and in such way representing a possible bias in risk assessment evaluations.

2.3.4 Existing guideline values

Biomonitoring equivalent (BE) for non-cancer effects (hyperpigmentation and vascular complications for humans) has been set by Hays et al., 2010 and is for chronic exposure 6.4 µg L^{-1}. BE_{POD} used as a starting point for the evaluation was 19.3 µg L^{-1} of total arsenic in urine, which includes the sum of iAs, MMA, and DMA (Hays et al. 2010). Above the BE value of 6.4 µg L^{-1} (which takes into account uncertainties, including inter-individual differences) health effects can't be excluded. Measured biomarker values above the human equivalent BE_{POD} indicate a high

priority for risk assessment follow-up (LaKind et al. 2008). BE values do not represent diagnostic criteria and cannot be used to evaluate the likelihood of an adverse health effect in an individual or even among a population. Values higher than the BE value are an indication for policymakers & risk managers and pinpoint the need to have a closer look at the risk assessment. BEs associated with the various cancer risk assessments are lower – BE of 1.4 µg L^{-1} is associated with cancer risk of 10^{-4} and 0.014 µg L^{-1} (urine) is associated with cancer risk of 10^{-6} (Hays et al. 2010).

2.4 Exposure assessment

2.4.1 Exposure assessment based on food intake calculations

iAs exposure was calculated for different population groups taking into account mean and P95 food consumption and different levels of iAs in food as explained in Chapter 2 (Methodology). Table 2 gives the data of calculated daily exposure to iAs via food and water expressed in µg kg-1 bw/day for seven age groups as average of countries, for which food consumption data were available.

Table 2: Summary of exposure to iAs (daily dose ± standard deviation) via food and water in EU (µg kg^{-1} bw/day)

Population category and no. of participants* in all countries		Calculated exposure to inorganic arsenic (iAs, µg kg^{-1} bw/day)					
		Mean food intake			P95 food intake		
		mean iAs	min iAs	max iAs	min iAs	mean iAs	max iAs
Infants	3713	0.20±0.07	0.11±0.05	0.38±0.13	0.30±0.12	0.64±0.21	1.23±0.42
Toddlers	3829	0.17±0.02	0.10±0.01	0.27±0.02	0.19±0.07	0.48±0.11	0.78±0.20
Other children	8885	0.12±0.01	0.08±0.01	0.18±0.03	0.21±0.04	0.33±0.05	0.46±0.07
Adolescents	8360	0.09±0.01	0.06±0.01	0.14±0.02	0.17±0.03	0.26±0.05	0.38±0.07
Adults	33752	0.08±0.01	0.05±0.01	0.12±0.01	0.14±0.02	0.23±0.03	0.35±0.04
Elderly	5098	0.08±0.01	0.05±0.01	0.12±0.01	0.13±0.02	0.21±0.03	0.32±0.04
Very elderly	2324	0.07±0.01	0.05±0.01	0.11±0.01	0.12±0.02	0.19±0.03	0.29±0.04

Standard deviation estimate is only based on differences in food intake between included countries.
*No. of participants relates to food consumption (FoodEx, 2016)

Our data was compared with previously published calculated daily doses for different population groups and good agreement was found with the calculations performed by EFSA (2021) while old report of EFSA (2014) gives considerably higher upper values.

2.4.2 Exposure assessment based on HBM data

Biomonitoring studies on larger populations without defined exposure to contaminants can confirm the exposure to measured substances, but are limited in identifying actual exposure sources, routes of exposure and the concentrations of arsenic in foods and the environment to which individuals are exposed to. For arsenic, a widely accepted marker of arsenic exposure is a concentration of iAs and its metabolites MMA and DMA in urine. The urine of the unexposed population contains low levels of arsenic, which is dominated by DMA. DMA is an end-product of iAs methylation in a body but can also be ingested with food as such (especially with rice and molluscs); avoiding seafood consumption for 3 days before urine sampling is advised. According to German standards, concentrations of total arsenic up to 15 µg L^{-1} are considered normal (Schulz et al. 2009), largely due to the expected presence of non-toxic arsenobetaine (Table 3). Elevated concentrations of total arsenic in the urine of the non-exposed population can almost always be ascribed to arsenobetaine ingested with seafood and excreted unchanged.

Table 3: Reference values for arsenic in blood and urine-based on Human Biomonitoring data

	Based on a study from	Time	Reference value ($\mu g\ L^{-1}$)	Reference
Total As in blood	Canada, teenagers	2007 – 2009	1.4	Saravanabhaven et al. 2017
Total As in blood	Canada, adults		2.0	
Total As in urine	Germany, children	2003 – 2006	15.0*	Schulz et al. 2009
Total As in urine	Germany, adults	1997 - 1999	15.0*	
i-As+MMA+DMA in urine	France		11	Garnier et al. 2020
i-As+MMA+DMA in urine	Australia		13 (unexposed) 15 (occupational)	SWA, Safe Work Australia 2002

*no fish consumption 48 hours before sampling

2.4.2.1 Determination of arsenic species in urine

The preferred sample for urine analysis is a 24-h urine sample, which minimises diurnal variability in analyte concentrations. However, because of practical considerations, a first-morning void or a random spot urine sample is often collected. In a study of families in Utah exposed to As in drinking water, the As concentrations in urine expressed as micrograms per gram creatinine were relatively stable throughout the day (Calderon et al. 1999). Adjustment to specific gravity or creatinine corrects for dilution (Hsieh et al. 2019). Specific gravity performed better than correction to creatinine in a recent study by Middleton et al. (2019). In a population with low-level environmental As exposure, it was reported that unadjusted and creatinine-adjusted urinary concentrations of inorganic As were significantly correlated (Hinwood et al. 2002). The investigators concluded that creatinine adjustment was not necessary for such a population.

Arsenic speciation requires specialised knowledge and equipment not commonly available in routine analytical laboratories. Ability to detect and accurately quantify a range of toxicologically relevant arsenic species is common to several analytical techniques with high-performance liquid chromatography (HPLC), which separates the compounds of interest before detection with ICP-MS or HG-AFS, being the base of most of them (Nearing et al. 2014, Ali & Jain 2004, B'Hymer & Caruso 2004). Several Standard Reference Materials (SRMs) are available for arsenic speciation in urine.

2.4.2.2 Results of available HBM studies

Table 4 gives an overview of recent HBM studies on the total arsenic in blood and urine in Europe for populations without known exposure to arsenic. Low excretion of arsenic in urine is evident from geometric means for the total arsenic (3.42 – 12.9 $\mu g\ L^{-1}$ for most of the studies, 18.2 $\mu g\ L^{-1}$ in one study from France). For comparison, Table 5 lists a few studies of populations with known exposure to arsenic in Italy, Poland, and other parts of the world, where much higher levels of the total and iAs and its metabolites were found in urine samples.

Table 4: Concentrations of total arsenic in blood and urine according to recent findings of HBM studies on populations without known arsenic exposure sources

Country, study and year, *number of participants*	As_{total} blood ($\mu g\ L^{-1}$)	As_{total} urine ($\mu g\ L^{-1}$)	Reference
Belgium, women, FLEHS 2007-2011, *235*	0.64 GM 2.04 P90		Schoeters et al.2012
Belgium, adolescents, FLEHS 2007-2011, *207*	0.62 GM 2.12 P90		Schoeters et al. 2012
France, IMEPOGE 2008-2010, *2000*	1.67 GM 6.72 P95	18.2 GM 131 P95	Nisse et al. 2017
France, adults, ENNS 2006-2007, *1515*		12.0 GM	Frery et al. 2012, Saoudi et al. 2012, Garnier et al. 2020
Germany, adults GerESIII 1998, *4052*	0.61 GM 2.4 P95	3.87 GM 19.3 P95	Kolossa-Gehring et al. 2012
Italy, adolescents, PROBE 2008-2010, *252*	0.82 GM 3.69P95		Pino et al. 2012
Serbia, adults, 2018, *305*	0.50 GM 1.70 P95		Stojsavljević et al. 2019
Slovenia, adults, 2008-2014, *1084 (blood), 812 (urine)*	0.89 GM 3.74 P95	6.37 GM 54.2 P95	Snoj Tratnik et al. 2019
Slovenia, adults Pilot HBM 2007-2009, *274*	0.74 GM 2.98 P95		Snoj-Tratnik et al. 2012
Slovenia, women, PHIME-FP6, 2006-2011, *176*	0.65GM	3.42 GM	Stajnko et al. 2019
Spain, adults, Andalusia, 2014-2015, *419*	1.39 male* 1.62 female*		Henríquez-Hernández et al. 2020
Italy, children, 2020, *200*		12.9 GM 104 P95	Bocca et al. 2020

*mean concentration in plasma

Table 5: Concentrations of total arsenic in blood and urine according to recent (after 2000) findings of HBM studies of populations with known occupational or environmental arsenic exposure

Country and year, number of participants	Total As blood ($\mu g\ L^{-1}$)	Total As urine ($\mu g\ L^{-1}$)	Reference
Italy, (2013-2014) industrial areas, 1177 adults		17.3 – 20.3 GMs	Ancona et al. 2016
Italy, Taranto, polluted area, 299 children		8.3 ME	Lucchini et al. 2019
Poland, (2001), 65 smelter workers 71 control		54.0 ± 42.3 11.0 ± 10.8	Lewińska et al. 2007
Spain, petrochemical refinery, 144 adults	22.4 ± 8.3		Ferré-Huguet et al. 2009
Spain, Huelva, industrial area, 261 children		2.43 GM 20.78 P95	Molina-Villalba et al. 2015
Croatia, close to the petrochemical industry, 20 adults	2.10-3.20 ME	4.16–5.77 ME	Cvitković et al. 2017

In Table 6, recent HBM studies published after 2010 from the EU and studies from this project are given. Average value of GM data for iAs and its metabolites is 0.16 ± 0.07, which also includes two sets of data from Spain, study 26, 9.15 $\mu g\ L^{-1}$ and study 27, 6.61 $\mu g\ L^{-1}$, both high above results of other studies. P95 urinary iAs and its metabolites are in the range of 4.7 – 14.7 $\mu g\ L^{-1}$ (0.48 ± 0.19), again including two higher P95 values found in the same two studies from Spain (study 26, 23.9 $\mu g\ L^{-1}$ and study 27, 21.5 $\mu g\ L^{-1}$). In a study 26 of Yusa et al. (2018) highest GM and P95 iAs+MMA+DMA concentrations were found and authors themselves suggested that additional DMA must have come from seafood since its content in urine was higher as expected.

Daily iAs exposure doses (Tables 6 and 7) were calculated according to Hays et al. (2010) as given in Chapter 2 from the urinary concentrations of iAs and its metabolites.

Table 6: Urinary concentrations of iAs and its metabolites according to recent findings of HBM studies of populations without known exposure to arsenic, and a daily dose of iAs back-calculated (reverse dosimetry) from urinary iAs+MMA+DMA according to Hays et al. 2010.

Non-exposed populations, Country, study, date, and *No. of participants*	Urinary iAs+MMA+DMA (measured, µg L^{-1} or in µg $g^{-1}_{creatinine}$* when marked with*)		Daily dose iAs, (µg kg^{-1} bw/day, calculated according to Hays et al. 2010)		References
	GM	P95 or else	GM	P95 or else	
1 - Belgium, FLEHS 2, 2008-2009, 194 adults	4.0	11.5 P90	0.17	0.48 P90	Schoeters et al. 2012
2 - Belgium, FLEHS 2, 2008-2009, 203 adolescents 14-15y	4.8	10.8 P90	0.20	0.45 P90	
3 - Belgium, FLEHS 3, 2013, *207* adolescents 12-19y	4.05 ME	14.7	0.17	0.61	HBM4EU Dashboard[$]
4 – Belgium, FLEHS pooled 1999-2018, 1322 adolescents	2.80*	4.02* P75	0.09	0.13 P75	Koppen et al. 2020
5 – Belgium, T_VITO_FLEHS IV, 2021, 148 adolescents	4.02	10.51	0.17	0.43	HBM4EU repository
6 – Belgium, 3xG, birth cohort (mother) 2010-2014, 151 women	4.34 (4.25 ME)	10.5	0.18	0.43	HBM4EU Dashboard[$]
7 - Croatia, PHIME-FP6, 2006-2011, 136 pregnant women	3.23	14.5	0.13	0.60	Stajnko et al. 2019
8 - Czech Republic, 384 children 8-10 years and adults	3.5* ME	12.1*	0.11 ME	0.39	Spěváčková et al. 2002
9 - France, ENNS 2006-2007, 1515 adults	3.34*	8.9*	0.14	0.37	Frery et al. 2012, Saoudi et al. 2012
10,11 - France, elevated As in soil, contamination not confirmed, 29/23 children (2-6 years) (summer/winter)	2.8 3.7	6.5 6.1	Calculation not performed[#]		Fillol et al. 2013
12 - Germany, children and adolescents, GerES IV 2003-2006, *173, (3-14 years)*	4.4 ME	14.0	0.18[##]	0.58[##]	HBM4EU Dashboard[$]
13 – Germany, GerES V, 300 adolescents	3.93	11.63	0.16	0.48	HBM4EU repository
14 - GerES V, 1266 adolescents (10 – 17 years), 2015-2017	2.81*		0.09		Zimmermann et al. in preparation
15 - GerES V,1028 children (3 – 9 years), 2015-2017	4.93*		0.16[##]		Zimmermann et al. in preparation
16 - Ireland, NICOLA, 89 adults over age 50	3.54	9.23	0.15	0.38	De Moraes et al. 2020
17 - Italy, 2020, 200 children, 7 years	4.26	13.4	0.19	0.55	Bocca et al. 2020
18 – Northern Ireland, 11 infants pre-weaning	0.57		Calculation not performed[#]		Signes-Pastor et al. 2017
19 - Northern Ireland, 11 infants post-weaning	2.81				

Non-exposed populations, Country, study, date, and *No. of participants*	**Urinary iAs+MMA+DMA (measured, µg L^{-1} or in µg $g^{-1}_{creatinine}$* when marked with*)**		**Daily dose iAs, (µg kg^{-1} bw/day, calculated according to Hays et al. 2010)**		**References**
	GM	**P95 or else**	**GM**	**P95 or else**	
20- Slovenia, CROME-LIFE+, 2013-2016, 174 women	1.83	12.2	0.08	0.50	Stajnko et al. 2019 + unpublished data from the same study
21 - Slovenia, CROME-LIFE+, 2013-2016, 174 children	2.17	12.8	0.09	0.53	
22 – Slovenia, CRP Prekmurje, 129 children	3.60	9.14	0.15	0.38	Snoj Tratnik et al. 2019
23 – Slovenia, CRP Prekmurje, 94 adolescents	3.66	10.6	0.15	0.44	Snoj Tratnik et al. 2019
24 - Slovenia, elevated As in soil, contaminated area not different from control area, 2016, 154 children (3-12 years)	2.72	7.3	0.11##	0.30##	Perharič et al. 2017
25 - Spain, industrial area, Andalusia, 2010, 196 children and adolescents 5-17 years	1.33	4.7	0.05##	0.19##	Aguilera et al. 2010
26 – Spain, Valencia, 109 children, age 6-11 – authors indicate possibility of additional dietary source of DMA	9.15	23.9	0.38	0.99	Yusà et al. 2018
27 – Spain, ISCIII_BEA, 300 adolescents	6.61	21.53	0.27	0.89	HBM4EU repository
28 – Sweden, Riksmaten Ungdom, 300 adolescents	5.42	12.26	0.22	0.51	HBM4EU repository
The average of EU data for all except infants###			0.16 ± 0.07	0.50 ± 0.19 (P95 only)	

P90 or P75 instead of P95 where explicitly mentioned - these data are excluded from a calculated average of P95 daily doses from different studies, ME – median instead of GM where explicitly mentioned, excluded from calculated average, *µg/$g_{creatinine}$,
** calculated by subtracting arsenobetaine and arsenocholine from the total As,
*** calculated by adding iAs+MMA+DMA,
#calculation not performed since the relationship from Hays et al. (2010) was determined for ages above 6 years
##calculated despite some of participants were younger than 6 years
average values are only a simple average of GMs of daily doses and do not consider the relative larger contributions of studies with more individuals or relative smaller contributions of studies with less individuals, other populations (e.g., does not appropriately represent all the age groups of a general population, etc.) and standard deviation is only showing the dispersion of calculated GMs or P95 daily dose values and does not show the actual dispersion or uncertainty of the estimated daily dose
$Dashboard European Human Biomonitoring Data for visualisation of aggregated data. Flemish Institute for Technological research (VITO), Mol, Belgium. URL https://www.hbm4eu.eu/what-we-do/european-hbm-platform/eu-hbm-dashboard/, consulted June 23 2021.

Table 7: Urinary concentrations of iAs and its metabolites according to recent findings of European HBM projects of populations occupationally or environmentally exposed to arsenic, and back-calculated daily doses (reverse dosimetry) of iAs (According to Hays et al. 2010).

Exposed populations, Country, study, date, and *No. of participants*	Urinary iAs+MMA+DMA (measured, µg L^{-1} or µg $g^{-1}_{creatinine}$*)		Daily dose iAs, (calculated, µg kg^{-1} bw/day, according to Hays et al. 2010)		Reference
	GM	P95	GM	P95	
Italy, (2010), SEpiAs, polluted area, *271* adults	8.76	86.28	0.4	3.4	Minichilli et al. 2018
Italy, Taranto, industrial area, 279 adults	6.1	16.8	0.25	0.69	Vimercati et al. 2016
Italy, Latium, 20 µg L^{-1} As in drinking water, 51 residents using this water for cooking and drinking	20		0.83		Cubadda et al. 2015
Poland, Cu smelter area, 2000 participants, speciation in 149 samples with total As > 15 µg L^{-1} (children and adults)	5.7 – 8.3	9.2 – 37.8	0.24 – 0.34	0.38 – 1.56	Kozłowska et al. 2019
Poland, copper smelter, 61 exposed 52 control	17.7 ME* 3.45 ME		0.73 ME 0.14 ME		Janasik et al. 2017
Slovakia, coal-burning power plant area 58 adults, 5 km radius 225 adults, 6-10 km distance 128 adults, >10 km distance	 7.4 6.0 5.5	 26.2 17.5 14.4	 0.31 0.25 0.22	 1.08 0.72 0.60	Wilhelm et al. 2005

*Median (ME) instead of GM where explicitly mentioned

2.4.3 Comparison of iAs daily doses obtained from food intake or from urine HBM data by reverse dose calculation

There are very little data available on iAs and its metabolites in the urine of the general population in Europe (without known As exposure sources). Studies collected in Table 6 include children, adolescents, and adults (pregnant women as well) and from Europe, only five of them include over 1000 participants. The data for Europe are not comparable – each population group has its characteristics, specific metabolism, and potentially different exposure. Nevertheless, the average daily dose calculated from these very diverse HBM data in Europe (GM) is very uniform, 0.16 ± 0.07 µg kg^{-1} bw/day (Table 6). Some specific subgroups, especially high rice consumers, are expected to have larger estimated As intake rates. Representativeness for the general EU population of the combined HBM studies cannot be assumed. Calculated average daily dose is in good agreement with daily dose calculated from food intake and iAs concentration data in food in Table 2 for mean food intake for adults (0.05 – 0.12 µg kg^{-1}/day) as well as children (0.08 - 0.18 µg kg^{-1}/day). For P95 consumption, calculation from food intake (adults, 0.23 – 0.35 µg kg^{-1}/day, children 0.33 – 0.46 µg kg^{-1}/day Table 2), also agrees well with HBM – calculated data (0.48 ± 0.19 µg kg^{-1}/day, Table 6, Figure 1).

From these calculations, we can be confident that there are no major »hidden« As exposures sources for the general population. Apart from study 26 performed in Spain (Yusà et al. 2018), with

an outlier with much higher iAs+MMA+DMA in the urine of children (and bigger data dispersion interval as indicated by P95), there is a noticeable absence of proof that children are exposed to higher daily doses of iAs compared to the other age groups. Average of GMs for studies involving children is 0.13 ± 0.04 µg kg^{-1}/day, P95 0.42 ± 0.14 µg kg^{-1}/day, excluding study 26, Table 6. For study 26 (Yusà et al. 2018), the authors indicated that the distribution among arsenic species was different as expected. According to (Hays et al. 2010), for GM of 9.15 µg L^{-1} of iAs+MMA+DMA, the theoretical concentration of DMA would be 5.03 µg L^{-1} (DMA = 55% of iAs+MMA+DMA) if it would be formed from iAs. However, they detected almost double the amount of DMA (8.32 µg L^{-1}) and very little of iAs (0.14 µg L^{-1}) and MMA (0.27 µg L^{-1}) and concluded that DMA could also come from direct ingestion and not only from methylation of iAs (Yusà et al. 2018).

Very few data are available for infants and toddlers. Signes-Pastor et al. (2017) studied a group of 79 infants in Ireland before and after weaning and found a significant increase in urinary iAs+MMA+DMA levels post-weaning (2.81 µg L^{-1}) compared to pre-weaning (0.57 µg L^{-1}) in a sub-group of 11 infants and connected the increase to rice-based solid foods. Similar conclusions were published for a group of 15 infants in the USA (Signes-Pastor et al. 2018).

Slightly higher HBM-calculated data are expected since dietary exposure calculations do not include an additional contribution of DMA from its direct ingestion or via degradation of ingested arsenosugars and arsenolipids (Francesconi et al. 2002, Schmeisser et al. 2006). Additional exposure is also expected for smokers (Minichilli et al. 2018, Brima et al. 2006, European Commission 2001), undetected occupational exposure, and exposure via air, soil, and dust. Except for rice and seafood, DMA concentrations in food are mainly unknown so the calculation of dietary exposure to DMA is highly speculative. One needs to be aware that additional dietary contribution of directly ingested DMA and DMA from the degradation of arsenosugars and arsenolipids is expected to be of less concern as exposure to iAs. Reasons being its much lower toxicity (Taylor et al. 2017, Kaise et al. 1989) and a different metabolic pathway. According to ATSDR (2007), the minimum risk of chronic exposure related adverse health effects is expected at 66 times higher concentrations as for iAs although a question of direct toxicity of different arsenosugars and arsenolipids remains unresolved. Lower toxicity of any form of arsenic in seafood can be supported by the lack of evidence on toxicity associated with significant seafood consumption (Luvonga et al. 2020, Borak et al. 2007). Analytical problems such as very low concentrations and several compounds below detection limit in many urine samples might also contribute to the overestimation of exposure based on HBM data. Therefore, HBM derived estimates of exposure likely overestimate the scale of iAs exposure, especially for higher (P95) data, which is expected to include more randomly present dietary DMA. Figure 1 illustrates the difference between daily doses of iAs calculated from dietary intake and from HBM derived data.

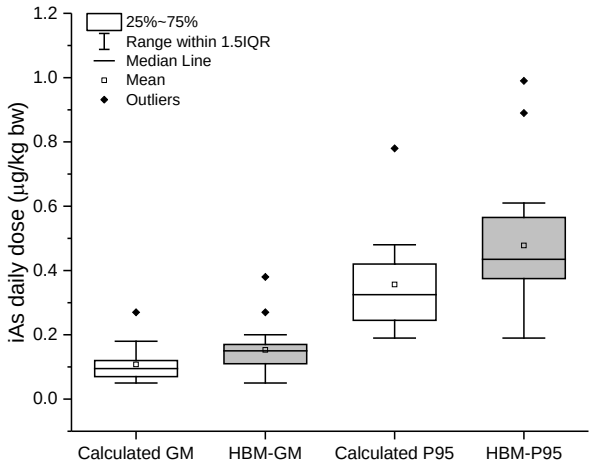

Figure 1: iAs daily dose (GM and P95), either calculated from food intake (Calculated GM and P95) or back-calculated (reverse dosimetry) from measured HBM studies (HBM-GM and HBM-P95) according to Hays et al (2010) including all European studies from Table 7 except infants

Calculated exposures to iAs of exposed populations are higher and more variable (GM 0.24 – 5.2 µg kg^{-1}/day, Table 7). The higher the concentration in a source of arsenic in an exposed population (drinking water or occupational), the less significant becomes the contribution from other sources (diet), which dominate in the case of unexposed population. In areas where arsenic levels in water are below 10 µg L^{-1} drinking-water guideline value, human effects are unlikely (JECFA 2011, Tsuji et al. 2019).

2.5 Risk assessment

2.5.1 Cancer risk assessment based on food intake data

Cancer risk was calculated with the use of no-threshold approaches and assuming linearity of dose-response curves, although it is recognised that this might overestimate the risk at low exposure levels due to adaptive responses (Tsuji et al. 2019, Cohen et al. 2019, Boffetta and Borron, 2019, Simon 2020). In addition, the assumption that the daily iAs dose would stay the same throughout lifetime is also bringing some uncertainty to the assessment – lifetime daily dose per kg of bw should consider the changing body weight in different life stages and relative duration of each exposure period. Apart from change in a daily dose (i.e. from infants to toddlers and further towards adulthood) also changes in efficiency and rate of metabolism are possible, and also the actual concentrations of iAs in drinking water, foods (or from other sources) are likely to vary during the period of around 80 years). These are, however, general uncertainties related to this kind of risk assessment approach. Because of these uncertainties instead of exact figures it is more important to consider the order of the magnitude of the risk.

When the daily dose and duration of exposure are taken into account, average excess lifetime cancer risk for adults, exposed to the estimated daily iAs concentration between the ages of 18 - 65 is $7.9 \cdot 10^{-5}$ (for lung cancer, based on ECHA 2013) and $7.0 \cdot 10^{-5}$ (for lung and bladder cancer, based on FDA 2016) with $2.3 \cdot 10^{-4}$ and $2 \cdot 10^{-4}$ for P95 respectively (Table 8).

Table 8: Excess lifetime cancer risk for different population groups derived from calculated iAs intake adopting the no-threshold approach, taking into account daily dose from Table 2 considering the duration of exposure for each population group.

Population	Mean food intake/mean iAs	P95 food intake/mean iAs
Lung cancer risk (JECFA 2011, ECHA 2013) = 1.7/1000 for 1 µg kg^{-1} bw/day		
Infants (0 - 1 year)	$4.2 \cdot 10^{-6} \pm 1.5 \cdot 10^{-6}$	$1.3 \cdot 10^{-5} \pm 4.4 \cdot 10^{-6}$
Toddlers (1 - 3 years)	$7.1 \cdot 10^{-6} \pm 1.8 \cdot 10^{-7}$	$2.0 \cdot 10^{-5} \pm 4.6 \cdot 10^{-6}$
Other children (3 -10 years)	$1.8 \cdot 10^{-5} \pm 1.6 \cdot 10^{-7}$	$4.8 \cdot 10^{-5} \pm 7.3 \cdot 10^{-6}$
Adolescents (10 – 18 years)	$1.5 \cdot 10^{-5} \pm 8.4 \cdot 10^{-8}$	$4.3 \cdot 10^{-5} \pm 7.3 \cdot 10^{-6}$
Adults (18 – 65 years)	$7.9 \cdot 10^{-5} \pm 4.9 \cdot 10^{-7}$	$2.3 \cdot 10^{-4} \pm 2.9 \cdot 10^{-5}$
50 years exposure - adult dose level	$8.4 \cdot 10^{-5} \pm 5.2 \cdot 10^{-7}$	$2.4 \cdot 10^{-4} \pm 3.1 \cdot 10^{-5}$
65 years exposure – contribution of different daily doses in different life stages	$1.2 \cdot 10^{-4} \pm 2.4 \cdot 10^{-6}$	$3.5 \cdot 10^{-4} \pm 5.4 \cdot 10^{-5}$
65 years exposure - adult dose level	$1.1 \cdot 10^{-4} \pm 6.8 \cdot 10^{-7}$	$3.1 \cdot 10^{-4} \pm 4.1 \cdot 10^{-5}$
Lung + bladder cancer risk (FDA 2016) = 1.506/1000 for 1 µg kg^{-1} bw/day		
Infants (0 - 1 year)	$3.7 \cdot 10^{-6} \pm 1.3 \cdot 10^{-6}$	$1.2 \cdot 10^{-5} \pm 3.9 \cdot 10^{-6}$
Toddlers (1 -3 years)	$6.3 \cdot 10^{-6} \pm 7.4 \cdot 10^{-7}$	$1.8 \cdot 10^{-5} \pm 4.1 \cdot 10^{-6}$
Other children (3 -10 years)	$1.6 \cdot 10^{-5} \pm 1.3 \cdot 10^{-6}$	$4.3 \cdot 10^{-5} \pm 6.5 \cdot 10^{-6}$
Adolescents (10 – 18 years)	$1.3 \cdot 10^{-5} \pm 1.5 \cdot 10^{-6}$	$3.9 \cdot 10^{-5} \pm 7.4 \cdot 10^{-6}$
Adults (18 – 65 years)	$7.0 \cdot 10^{-5} \pm 8.7 \cdot 10^{-6}$	$2.0 \cdot 10^{-4} \pm 2.6 \cdot 10^{-5}$
50 years exposure - adult dose level	$7.4 \cdot 10^{-5} \pm 9.3 \cdot 10^{-6}$	$2.1 \cdot 10^{-4} \pm 2.8 \cdot 10^{-5}$
65 years exposure – contribution of different daily doses in different life stages	$1.1 \cdot 10^{-4} \pm 1.4 \cdot 10^{-5}$	$3.1 \cdot 10^{-4} \pm 4.8 \cdot 10^{-5}$
65 years exposure - adult dose level	$9.6 \cdot 10^{-5} \pm 1.2 \cdot 10^{-5}$	$2.8 \cdot 10^{-4} \pm 3.6 \cdot 10^{-5}$

Next to iAs, no-threshold approach is currently revised for some other DNA-reactive and epigenetic experimental carcinogens (Kobets and Williams 2019) and low dose radiation induced health risks as well (Tharmalingan et al. 2019); the threshold level for iAs was estimated to be around 100 µg L^{-1} for drinking water (between 50 and 150 µg L^{-1}) (Cohen et al. 2019, Tsuji et al. 2019). An extensive study by Ferdosi et al. (2016) could find no link between lung cancer and drinking water arsenic (slopes indistinguishable from zero) in the range of 3 – 59 µg L^{-1} (population-weighted average 5 µg L^{-1}) for 133 US counties in years 1950 – 1979. The same was previously found by Lamm et al. (2004) on the same population for bladder cancer. In a long-term study, Zhang et al. (2016) followed over 200 000 men and women in the USA for 26 years. No link was found between rice consumption and any form of cancer in groups exposed to various levels of iAs consumed with rice.

2.5.2 Estimation of non-cancer effects based on HBM data

Biomonitoring equivalent (BE) for non-cancer effects for iAs for chronic exposure is 6.4 µg L^{-1} and BE_{POD} is 19.3 µg L^{-1} of total arsenic, which includes the sum of iAs, MMA, and DMA (Hays et al. 2010). Like other regulatory limits on iAs exposure, this values might also need an update.

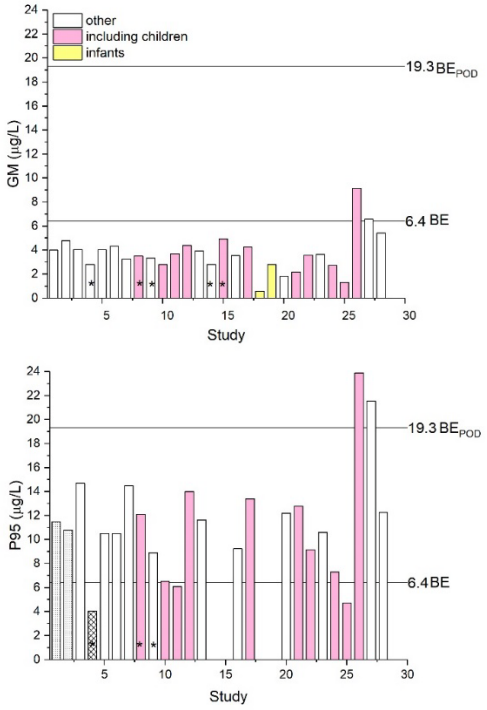

Figure 2: Inorganic As and its metabolites in urine in recent HBM studies from Table 6 compared to BE for non-cancer end points.*indicates sets of data given in µg/g creatinine. In studies 1 and 2 P90 data are shown (dotted) and in study 4, P75 instead of P95 is shown (crossed).

Apart from the study 26 most of the estimated geometric mean (GM) HBM levels fall within or below the BE value of 6.4 µg L^{-1} (Figure 2, Table 6). However, there are more studies where the estimated P95 levels are above BE values (and in two cases even above BE_{POD} values) showing that a risk of non-cancer health effects cannot to be excluded although in a study 26 of Yusa et al. (2018), where highest GM and P95 iAs+MMA+DMA concentrations were found, authors themselves suggested that additional DMA must have come from seafood since its content in urine was higher as expected.

In addition, it should be noted that this BE value is based on hyperpigmentation and vascular complications and do not necessarily fully cover all the adverse, non-cancer effects of arsenic. More research is necessary for cardiovascular effects seeing the associations found with low arsenic exposure in some studies (Xu et al. 2020, Medrano, 2010, Moon, 2014) while other studies were not able to identify any link between CVD and low levels of iAs (Sidhu et al. 2015, Eshak et al. 2014). Recent studies also indicate that arsenic is a developmental neurotoxicant and arsenic induced epigenetic changes are reported at higher levels (Tolins et al. 2014; Chakraborty et al 2022).

2.5.3 Cancer risk assessment based on HBM data

The average daily dose calculated from the HBM values (reverse dosimetry) from studies included in Table 6, is 0.16 µg kg^{-1} bw/day. The P95 level was 0.50 ± 0.19 µg kg^{-1} bw/day. The estimate is assuming the linear relationship between the steady state concentration of arsenic in urine and a daily dose of iAs (Hays et al. 2010). The pooled population of all the studies cannot represent other

populations and is not fully representative of the general EU population. It is also not representative for all age groups as the studies mostly focused on children and adolescents (cca. 80 % of the pooled population; Table 6). The gender representation for all age groups is also not assured and as given above As metabolism differs between female and male sex (Section 3.3). The estimated average value of daily iAs intake (0.16 kg^{-1} bw/day) includes also some uncertainties. The standard deviation of 0.07 (Table 6), indicating relatively large dispersion of GM values.

Using a non-threshold linear dose-response, with a lifetime excess lung cancer risk of $1.7 \cdot 10^{-3}$ per 1 µg kg^{-1} bw/day gives a cancer risk level of $2.7 \cdot 10^{-4}$ for the average exposure and $8.5 \cdot 10^{-4}$ for the P95 level. Noting the uncertainties related to the dose-response of arsenic, the calculated values must be interpreted with caution.

2.5.4 Uncertainties in the estimation of adverse health effects related to iAs exposure

Uncertainties related to exposure calculated from food intake

Of special importance is the limit of detection (LOD) problem. Most laboratories reported detection limits between 10 and 20 µg kg^{-1} for most foods, and of the approximately 100000 foods analysed in a study of EFSA, only 34 % were above the detection limit. From the newer data it seems that old lower bound estimates for food iAs are closer to the actual iAs concentrations than upper bound ones (e.g. EFSA 2009, EFSA 2021).

Other problems are difficulties in relevant estimations of oral bioavailability and biological effects from different food items or food mixtures. The bioavailability of iAs is high, although only a few hardly comparable studies exist (Li et al. 2017, Liao et al. 2020). Using a conservative approach assuming it to be 100 %, we should be aware that we are dealing with undefined overestimate. Uncertainty is also related to differences in animal-human As metabolism and physiology, even more important, to unknown interactions with additional food items in the same meal.

Not last, unreliable information of self-reported food intake together with a wide range of possible iAs content within the same food groups has to be considered as a source of uncertainty in the calculation of iAs intake (Scrafford et al. 2016).

Uncertainties related to HBM studies

Despite low detection limits and high precision of analytical methods for arsenic speciation, we need to be aware of potential errors with all analytical techniques. Bulka et al. (2017) made two estimates of exposure to iAs using the same set of measured urinary total arsenic and its metabolites in urine samples of 7398 participants. When arsenobetaine and arsenocholine were subtracted from the total As, 3.2 µg L^{-1} was calculated as toxicologicaly relevant content. Exactly double concentration was obtained (6.4 µg L^{-1}) by adding As(III), As(V), MMA and DMA concentrations. Differences between both estimates were not discussed in their publication.

Values below the detection limit are normally taken as 50 % of the detection limit (often the case for 3 out of four expected compounds) also contributing to a higher sum of metabolites. Normally the concentration of iAs and its metabolites is obtained by adding up concentrations of As(III), As(V), MMA, and DMA and, as indicated in this particular example, might yield questionable results.

Next to analytical considerations, confusing role in the estimation of dietary exposure to iAs as the most toxic arsenic species in food is played by DMA in food. DMA is a relatively common less toxic arsenic species present in higher concentrations especially in rice and in seafood. When ingested it is excreted unchanged in the urine. Since DMA is also an end product of iAs methylation and

degradation of arsenosugars and arsenolipids in the human body, its concentration in urine thus reflects recent ingestion of DMA, arsenosugars, arsenolipids, and iAs. From this point of view, DMA is not a reliable marker of iAs exposure as questioned by e.g. Navas-Acien (2011 and references therein).

Uncertainties are also related to the limited representativeness of individual or pooled HBM studies for general or any other population due to unrepresentative age structure, gender structure or other characteristics such as social determinants of health, genetic predispositions, etc.

Uncertainties related to iAs metabolism – linear versus nonlinear dose-response curves, threshold versus non-threshold approach, adaptation

In 2010, the Joint FAO/WHO Expert Committee on Food Additives (JECFA 2011) 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 µg L^{-1}, there is some evidence of adverse effects. In other areas, where arsenic concentrations in water are elevated (10 – 50 µg L^{-1}), 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 (JECFA 2011).

It is not clear yet if direct extrapolation from high concentrations predicts health effects at low concentrations (no threshold, Lanphear 2017). Such an approach was in the past and recently seriously questioned by Tsuji et al. (2019) and Cohen et al (2019); it seems that for such damage to occur sufficient concentration of arsenic is needed indicating that arsenic toxicity is a threshold process (Tsuji et al. 2019). Rhomberg et al. (2011) argue that there is no compelling evidence-based justification for general low-exposure linearity.

Based on the BMDLs calculated for cellular effects of trivalent arsenic mixtures representative of human internal exposures (Yager et al. 2013), the threshold for potentially adverse cellular effects from exposure to iAs in drinking water was considered to occur at urinary concentrations of trivalent arsenic above 0.2 µM (15 µg L^{-1}). This corresponds to drinking water total arsenic concentrations above 65 µg L^{-1}. Concentrations below this level were considered unlikely to result in adverse cellular effects, even after chronic exposure' (Tsuji et al. 2019). Recent biochemical and biologic research increasingly supports the conclusion that iAs-related cancer and non-cancer endpoints involve a threshold response (Cohen et al. 2013), although uncertainty of its exact level remains.

Although there are many epidemiological studies supporting a threshold (Sidhu et al. 2015, Lynch et al. 2017, Boffetta and Borron 2019), others show risks already at very low levels (Moon et al. 2013, Medrano et al. 2010, Xu et al 2020b). Therefore, no definitive conclusions on the presence or absence of threshold can be made based on epidemiological data.

On the other hand, smoking and simultaneous exposure to multiple contaminants from the environment and food, potentially increasing iAs toxicity, are normally not evaluated in risk estimations (Tsuji et al 2021).

2.6 Conclusions

Results of HBM studies confirm that European population is exposed to similar levels of iAs as estimated previously (EFSA 2021) and in this document additionally shown through the calculated exposure from food consumption. Subgroups with potentially higher exposure due to smoking, higher rice consumption or proximity of hotspots were not explicitly studied, but were also not excluded from studies included in this report. Direct ingestion of DMA, arsenosugars and

arsenolipids contributes to somewhat higher HBM-urine measured exposure and is one of the major sources of uncertainty in iAs risk assessment based on HBM studies.

Excess lifetime cancer risk estimation (for lung or lung and bladder cancer) was performed on the basis of calculated daily iAs intake (from food and HBM - urine data) and available cancer slopes for lung and lung + urinary bladder cancer. Excess lifetime cancer risk for adults is in the range of $9.6 \cdot 10^{-5}$ - $1.1 \cdot 10^{-4}$ for mean consumption and mean iAs levels and from $2.0 \cdot 10^{-4}$ – $3.1 \cdot 10^{-4}$ for P95 food consumption and mean iAs levels taking into account different scenarios and life-long exposure (Table 8). The estimation includes uncertainties as discussed above.

Remark from the working group: This report tries to present the results in a neutral manner. The report preparing group (Šlejkovec, Falnoga, Bizjak and Horvat) and the wider arsenic group (Buekers, Mahiout, Fletcher, Uhl, Meslin, Roussele, Schoeters, Bessems, Santonen) were unable to reach consensus

- About the adequacy of scientific data supporting non-linear (threshold) approach.
- About poor relevance of calculated cancer risk estimation based on outdated, although still valid, risk-slopes (ECHA 2013, FDA 2016) which are under revision. US EPA is preparing a new risk assessment guidance with opening the new possibilities for using new epidemiological low-exposure data and different approaches in arsenic health risk assessment (including hierarchical, Bayesian meta-analysis approach combined with sensitivity analysis) (US EPA, 2019; National Academies of Sciences, Engineering and Medicine, 2019).
- About cardiovascular effects at low level exposure, for which contradictory reports exist
- About the need for further HBM studies of unexposed populations.

Report preparing group stands on position that current scientific information related to low levels of iAs exposure could not be overlooked (analytical uncertainties, urine DMA as a misleading indicator for low iAs exposure, non-linearity, threshold, homeostasis, adaptation, mode of action, etc). Considering these issues, thoroughly discussed throughout report, the estimated cancer risk assessment is of poor relevance, prediction is overestimated and there is no need for regular HBM studies for healthy nutritional uncompromised non-smoking adult populations. It should be noted that we can not disagree with a recent textbook of Simon (2020, Environmental risk assessment: a toxicological approach) as a whole and with a statement that a ‘low-dose linearity is an expression of the precautionary principle and is inconsistent with the accumulated knowledge of biology’.

Results in the light of policy questions

1. What is the current exposure of the EU population to arsenic? On the basis of external exposure, calculated from iAs intake from diet (mean intake from diet, min – max iAs levels), EU population is exposed to: 0.05 – 0.14 µg kg-1 bw/day (adolescents, adults, elderly and very elderly) or to 0.08 – 0.38 µg kg-1 bw/day (infants, toddlers, other children). P95 food intake results in higher exposure (0.19 – 0.38 µg kg-1 bw/day for adolescents, adults, elderly and very elderly and 0.33 - 1.23 µg kg-1 bw/day for infants, toddlers and other children). HBM based estimates are in the same range – calculated average dose of all included HBM studies (excluding infants) is 0.16 µg As kg-1 bw/day. HBM data included in the study did not identify any (previously unknown) higher levels of iAs exposure in the general population.
2. What biomonitoring and exposure (environmental and occupational) data on arsenic, relevant to the European population, are currently available? We identified 28 sets of data on populations without known sources of iAs exposure and four studies for occupationally exposed populations, all reporting speciated urinary iAs and its metabolites concentrations.

3. What is the geographic spread of the current exposure and how does it relate to different exposure sources (environmental; dietary sources)?
   Due to low iAs concentrations in drinking water in most EU countries (GM 0.19 µg L^{-1}, P75 0.47 µg L^{-1}, 99 % of samples below the maximum permissible value of 10 µg L^{-1} Banks et al. 2015), high exposure is limited to few areas with elevated As in drinking water (some areas in Italy, Hungary, Slovakia, Romania, Finland, Ireland, Greece, Croatia). For the majority of the population, diet is a main source of iAs. Calculated exposure is somewhat higher for populations that consume more seafood and/or rice.
4. Which population groups are most at risk?
   a) Infants followed by toddlers and other children consume most iAs per kilogram body weight, nevertheless they are also faster As metabolisers so such straightforward estimation of higher risk should be taken with caution, especially when short duration of higher exposure (per kg of body weight) is taken into account.
   b) Populations in the areas with elevated As in drinking water are at risk or occupationally exposed individuals.

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?
   Genetic polymorphisms related to metabolic rate of As (ASMT3 polymorphisms) are important as adaptation mechanisms leading in resistance to arsenic toxicity, while MTHR polymorphisms influencing folate levels could be involved in increased susceptibility to As toxicity, particularly during pregnancy with concomitant folate deficiency.
   Urinary DMA is widely included as one of the markers of iAs exposure. However, it can also be excreted in urine after direct ingestion or after ingestion of less toxic arsenosugars and arsenolipids hence overestimating the exposure to iAs. Better markers are iAs and MMA, but their concentration is often so low that analytical considerations are an issue.
6. What are possible health effects resulting from chronic low exposure to arsenic from food consumption?
   At long-time exposure to high iAs levels, lung, bladder and skin cancers are the most researched and confirmed outcomes. Extrapolation estimated daily dose of iAs from HBM studies (0.16 µg kg-1 bw/day) results in a lifetime excess lung cancer risk of 2.7 x 10-4. This number is of the same order of magnitude as previously dietary intake calculated by EFSA based on food intake and occurrence levels in food. However, it should be noted that since the risk assessment is based on the assumption of a linear relationship between exposure and cancer risk, this approach can be considered as conservative, overestimating the risk at low exposure levels. Currently, the scientific community have not agreed whether it is possible or not to identify a threshold for the carcinogenicity of arsenic. Evaluation of the most recent dose-response data and justification of assumptions behind EFSA's dose-response, necessary to assure the currency of the dose-response, was beyond the scope of this work, further emphasising that the calculated risk levels must be interpreted with caution. Additional uncertainty is related to the representativeness of populations and applicability of epidemiological data, overestimation of iAs exposure in HBM studies due to the widespread presence of DMA in food, analytical challenges related to speciation of As and inter-individual differences, including individual lifestyle and susceptibility factors. Research group preparing full arsenic report and reviewers of the report did not reach consensus on applicability of existing cancer slopes derived from high exposures to low exposures and a possible threshold for arsenic toxicity; differing views concerned

specifically whether negative health effects, including cancer, are relevant at exposure levels < 50 µg L-1 in drinking water.

7. What are the best analytical methods should allow for differentiating species in urine?

Most sensitive and most widely-available analytical methods include chromatographic separation of iAs, MMA and DMA and determination of As in separated peaks with ICP-MS or HG-AFS.

Future prospects

As discussed above, at this moment, there is no consensus of cancer risk of iAs in low-level exposure.

Several data are still required in a near future to allow a more accurate risk assessment:

- HBM data are missing on iAs exposure of populations consuming polluted water with As concentrations above 10 µg L^{-1} and populations in polluted hot-spots.
- Relevant covariates (e.g. smoking, selenium nutritional status, co-exposures with other pollutants, abstinence from rice and seafood intake before sampling, past exposures) should be controlled in future HBM studies (Tsuji et al. 2021).
- Guideline values need to be harmonised.

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3 Aprotic solvents full RA report

Risk assessment for aprotic solvents: NMP, NEP and DMF

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3.1 Summary

Because of the toxicological profile, a risk assessment for the four aprotic solvents N-methyl pyrrolidone (NMP), N-ethyl pyrrolidone (NEP), N,N-dimethylacetamide (DMAC) and N,N-dimethylformamide (DMF) was planned to be done for all 4 substances. These solvents have a similar toxicological profile and a harmonised classification for reproductive toxicity under the CLP regulation (Repr. 1B). Due to their wide use and high production volume in Europe, a high frequency of exposure is expected.

Even though these substances are of high toxicological concern, a literature search revealed the scarcity of HBM data for these substances for the European population. For DMAC data could only be found for highly exposed workers, for DMF samples from the German Environmental Specimen Bank (ESB) were analysed for the metabolite AMCC for the years 2000 to 2021 (data unpublished). Additionally, some data are available from the control group in an occupational study from Germany (Kilo et al., 2016).

For NMP and NEP exposure data are available from two studies conducted in Germany. These include data from the ESB taken from 1991 to 2014 (Ulrich et al., 2018) and data from the German Environmental Survey of Children and Adolescents V (GerES V) (Schmied-Tobies et al., 2021). The data of the two studies were taken as the basis for the risk assessment for NMP and NEP.

The samples from the ESB allow investigation of time trends for NMP, NEP and DMF.

Within HBM4EU, Human Biomonitoring Guidance Values for the general population (HBM-GV_{GenPop}) have been derived for NMP and NEP. For children this value is 10 mg/L for both NMP and NEP. For adolescents and adults an HBM-GV_{GenPop} adolescents, adults has been derived of 15 mg/L both for NMP and NEP. For DMF a provisional HBM-$GV_{Workers}$ has been derived for the metabolite AMCC of 10 mg/g creatinine. For the purpose of this risk assessment this value was adjusted to a provisional HBM-GV_{GenPop} of 1 mg/g creatinine for a comparison with the data from ESB. The key steps of the derivation of these values are presented.

The key exposure data from the studies from Germany are presented and compared.

A comparison of the exposure data with the newly derived HBM-GV_{GenPop} showed that exposure for adults (ESB), children (GerES V) and adolescents (GerES V) is well below the Guidance Values both for NMP and NEP. Maximum values of the studies were a factor of 4.7 to 10 lower than the corresponding HBM-GV_{GenPop} values. The maximum value found in the data from ESB for the DMF metabolite AMCC was a factor of 2.5 lower than the provisional HBM-GV_{GenPop} of 1 mg/g creatinine.

Even when considering the combined exposure to NMP and NEP, the values are not exceeded. The calculated hazard index (HI) was well below 1 in all cases considered (i.e., children, adolescents and adults) with maximum HI values of 0.3, indicating that there was no exceedance of the HBM-GVs. For young adults the HI was calculated for the combined exposure to NMP, NEP and DMF resulting in a maximum HI value of 0.6. However, a possible combined exposure with other reprotoxic substances present in the environment should be considered in “real-life-situations”, since these might increase the risk for common effects (Kortenkamp and Faust, 2018).

The high percentage of values above the limit of quantification in the two studies from Germany, and the newly analysed samples for the DMF metabolite, clearly shows that the investigated population was exposed to NMP, NEP and DMF.

The analysis of time trends of exposure towards NMP and NEP (years 1991-2014) revealed a continuous exposure towards both NMP and NEP over the time span investigated. For DMF (years

2000-2021) a > 50% decrease of AMCC concentrations could be observed for the time span investigated.

The analysis of the data from the GerES V study showed that for NMP, highest exposure was found in young children, but exposure pathways were not possible to be revealed. Exposure to NEP was highest in adolescents and participants with low socio-economic status or migration backgrounds. Associations to usage of personal care products suggested that the choice of products had a distinct impact on NEP exposure.

The original goal of including DMAC also was not realised due to a lack of HBM data for the general population. Therefore, monitoring for DMAC metabolites in the European population is highly recommended, including susceptible subpopulations to broaden the database. The sources of exposure for the aprotic solvents need to be further investigated and clarified.

Introduction

The four aprotic solvents, N-methyl pyrrolidone (NMP), N-ethyl pyrrolidone (NEP), N,N-dimethylacetamide (DMAC) and N,N-dimethylformamide (DMF) have a similar toxicological profile and a harmonised classification for reproductive toxicity under the CLP regulation (Repr. 1B, H360D – May damage the unborn child). Since they are widely used, have a wide range of applications and have a high production volume in Europe, a high frequency of exposure is expected. The original goal of the assessment for aprotic solvents was to answer the policy-related research questions described in the Scoping Document of aprotic solvents (Kadikis, 2020) within HBM4EU which comprises several aims:

- Building a picture of internal exposure burden from NMP and NEP within Europe based on available HBM data;
- Gaining more knowledge on the most vulnerable and highly exposed population groups;
- Evaluating correlations of internal exposure with lifestyle behaviours and usage of certain products;
- Assessing if internal exposure exceeds available HBM Guidance Values for the aprotic solvents;
- Evaluating risks of combined exposure of the reprotoxic aprotic solvents (at least for NEP and NMP; whether this is also possible for DMAC and DMF shall be explored).

However, a literature search on exposure data for aprotic solvents revealed that not many data are available for the general population in Europe. For NMP and NEP Human Biomonitoring data can be found in two studies from Germany. Consequently, for the risk assessment the HBM data from Germany have been used as a basis for this task. The available data are compared with the newly derived HBM-GV_{GenPop} for NMP and NEP, thereby also considering time trends in the exposure.

For DMF some data are available from an occupational study in Germany (Kilo et al. 2016) for the metabolite AMCC (N-Acetyl-S-(N-methylcarbamoyl)cysteine) in urinary samples of unexposed workers. For the risk assessment and assessment of time trends urinary samples from the German Environmental Specimen Bank (ESB) from the year 2000 to 2021 were analysed for the metabolite AMCC in a total of 360 samples (urinary samples of 30 male and female students each at 6 different time points within the time frame from 2000 to 2021).

These data are compared with the newly derived HBM-GV_{Worker} after an adjustment of this value for the general population (HBM-GV_{GenPop}). The provisional $HBMGV_{GenPop}$ was derived for orientation purposes to enable the assessment of the contribution of DMF to the total risk of the exposure towards the aproctic solvents considered.

For DMAC data can only be found for highly exposed workers (details are given in the Annex).

Knowledge gaps that need to be filled for an improved risk assessment are discussed.

3.2 Methodology

The document will follow the outline as presented below:

Chapter 3: In the hazard assessment chapter, we present general information on NMP, NEP, DMAC and DMF and give an overview on physico-chemical properties of the four substances (for details please see the Scoping document for aprotic solvents (Kadikis, 2020). For each substance we present the key steps for the derivation of the HMB-GVs used for the later risk assessment.

Chapter 4: For the exposure assessment a literature search via PubMed and Scopus was conducted to search for available biomonitoring data. The literature search was completed on 25th January 2021. For NMP and NEP, HBM data are available from the German Environmental Survey V (GerES V) for children and adolescents (Schmied-Tobies et al., 2021) and from the German Environmental Specimen Bank (ESB) for students aged 20-30 years (Ulrich et al., 2018). From the ESB study (Ulrich et al., 2018) data are also available for the years 1991-2014, which allows for the investigation of time trends.

After a brief description of uses and potential exposure to the solvents, the main findings of the two publications with HBM data from Germany are presented and discussed.

For DMAC Human Biomonitoring data are only available for the occupational population (see Annex Tables A8). Following, for DMAC no risk assessment for the general population can be carried out.

For DMF samples from the German Environmental Specimen Bank (ESB) were analysed in a total of 360 samples for the metabolite AMCC for students aged 20-30 years. Data are available for the years 2000-2021, which allows investigation of time trends. The aggregated data for the metabolite AMCC are unpublished. All values were > LOQ. Additionally, some data are available from an occupational study in Germany for urinary AMCC levels in non-exposed workers (Kilo et al., 2016).

Chapter 5: In the risk characterisation chapter, the exposure data found are compared with the newly derived HBM-GV_{GenPop} values for NMP and NEP for the following age groups: children (3-13 years), adolescents (14-17 years) and young adults (20-30 years). In a second step the risk of combined exposure to NMP and NEP is assessed applying the Hazard Index (HI) approach separately for children, teenagers and young adults.

For DMF the newly derived provisional HGM-GV_{Worker} for DMF was adjusted for use for the general population and compared with the exposure data from the German ESB for the metabolite AMCC. In a second step the risk of combined exposure to NMP, NEP and DMF was assessed for young adults.

Chapter 6: Finally, in the conclusion chapter, basic statements - with regard to the stated policy questions - are summarised and open questions are addressed as well as identified knowledge gaps are listed.

Detailed overview on the aprotic solvents in question is given in the Scoping document (Kadikis, 2020).

3.3 Hazard assessment

3.3.1 General information and physico-chemical properties

The general information on the four aprotic solvents is given In the Table 1. In addition, physico-chemical properties of NMP, NEP, DMAC and NMF are presented in the Table 2.

Table 1: General information on NMP, NEP, DMAC and NMF (acc. to ECHA brief profile, accessed 17.02.2021)

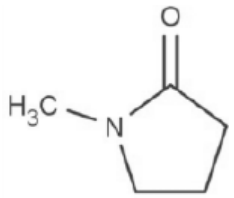
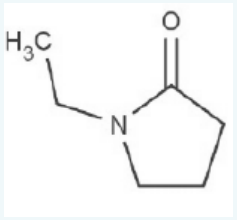
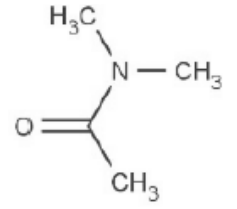
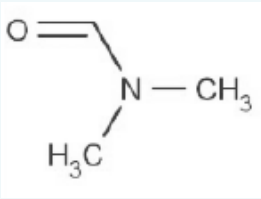
Abbreviated name	CAS number	EC number	IUPAC name	Molecular weight	Molecular formular	Molecular structure	Harmonised CLP classification
NMP	872-50-4	212-828-1	1-methylpyrrolidin-2-one	99.13 g/mol	C5H9NO		Repr. 1B (H360D) Skin Irrit. 2 (H315) Eye Irrit. 2 (H319) STOT SE 3 (H335)
NEP	2687-91-4	220-250-6	1-ethylpyrrolidin-2-one	113.16 g/mol	C6H11NO		Repr. 1B (H360D)
DMAC	127-19-5	204-826-4	N,N-dimethylacetamide	87.12 g/mol	C4H9NO		Acute Tox. 4 (H312) Repr. 1B (H360D) Acute Tox. 4 (H332)
DMF	68-12-2	200-679-5	N,N-dimethylformamide	73.09 g/mol	C3H7NO		Repr. 1B (H360D) Acute Tox. 4 (H312) Eye Irrit. 2 (H319) Acute Tox. 4 (H332)

Table 2: Physico-chemical properties of NMP, NEP, DMAC and NMF (acc. to ECHA brief profile, accessed 17.02.2021)

Substance	State at room temperature	Melting point	Boiling point	Relative density	Vapour pressure	Partition coefficient log K_{ow}	Water solubility
NMP CAS 872-50-4	liquid, colourless (slightly yellowish)	-24.2 °C (101.3 kPa)	204.3 °C (101.58 kPa)	1.03 g/cm³ (25 °C)	32 - 254 Pa (20 - 50 °C)	-0.46 (25 °C)	1 000 g/L (20 °C)
NEP CAS 2687-91-4	liquid, colourless (slightly yellowish)	-120 - -100 °C	212.5 °C (101.325 kPa)	0.997 (20 °C)	18 - 165 Pa (20 - 50 °C)	-0.2 (23 °C, pH 7 - 7.4)	1 000 g/L (23 °C, pH 9 – 12)
DMAC CAS 127-19-5	Liquid, colourless	-20 °C	166 °C (101.325 kPa)	0.94 g/cm³ (20 °C)	2 hPa (21.7 °C)	-0.77 (25 °C)	1 000 g/L (20 °C)
DMF CAS 68-12-2	liquid, colourless, (light yellow)	-61.4 - -60.5 °C	152 - 153.5 °C (101.3 kPa)	0.94 - 0.95 g/cm³ (20 - 25 °C)	3.08 - 3.77 hPa (20 °C)	-1.01 - -0.85 (25 °C)	1 000 g/L (20 °C)

3.3.2 Dose-response: Derivation of Human Biomonitoring Guidance Values (HBM-GVs) for NMP, NEP and DMF

Within the framework of Task 5.2 of HBM4EU, Human Biomonitoring Guidance Values (HBM-GVs) were derived for some prioritised substances.

HBM-GVs derived for the general population (HBM-GV_{GenPop}) are defined as the concentration of a substance or its specific metabolite(s) in human biological media (e.g., urine, blood, hair) at and below which, adverse human health effects, according to the current knowledge, are not to be expected (Apel et al., 2020).

HBM-GVs for NMP and NEP were derived for the general population (David et al., 2021). For the rationale behind the derivation of these values see Task 5.2 Deliverable Report D 5.9. In the present document the derivation of the HBM-GVs for NMP and NEP is merely briefly described.

3.3.2.1 Derivation of HBM-GVGenPop for NMP

NMP is classified as a skin and eye irritant. In animal studies at high doses effects on body weight, liver, kidney, spleen and thymus were observed. Developmental toxicity is considered as the most sensitive effect for human health risk assessment (NICNAS, 2018). There are no epidemiological studies investigating the health effects of NMP in humans.

- Choice of biomarkers of exposure for NMP

The analysis of the two metabolites 5-HNMP (5-hydroxy-N-methyl-2-pyrrolidone) and 2-HMSI (2-hydroxy-N-methylsuccinimide) as substance-specific biomarkers of exposure for NMP in urine is recommended (Deutsche Forschungsgemeinschaft (DFG), 2008; Käfferlein et al., 2013).

- Choice of the key study and application of assessment factors

As Apel et al. 2020 outlined in their strategy paper on the derivation of health-based HBM Guidance Values, the selection of the key study shall include the exposure pathway that is also considered as the relevant exposure pathway for humans.

For NMP the dermal route of exposure is thought to be the most relevant route of exposure before inhalation exposure. The oral route of exposure is secondary due to the areas of application. However, the derivation of an HBM-GV on the basis of animal studies with dermal administration is considered too uncertain due to the difficulties in extrapolation, particularly with regard to differences in dermal absorption between humans and rodents. Concerning the available studies with whole-body inhalation exposure, uncertainties exist in relating the NMP concentration in the air to the internal body burden, and as well as with respect to additional dermal and oral intake. Therefore, the oral developmental toxicity study by Saillenfait et al. (2002) on rats was considered as the key study for derivation of HBM-Gvs.

In the study in question, pregnant Sprague-Dawley rats were given NMP at doses of 0, 125, 250, 500 and 750 mg/kg/d by gavage, on gestational days 6 through 20. In summary, the no-observed-adverse-effect level (NOAEL) for maternal and developmental toxicity was 250 and 125 mg/kg/d, respectively. Thus, oral administration of NMP produced developmental toxicity below maternal toxicity levels.

Additionally, the oral study of Sitarek et al. (2012) on reproductive and developmental toxicity was regarded as being essential for the derivation of HBM-GVs showing effects on offsprings (reduced survival rate) and maternal animals (reduced body weight) at all doses applied. In this study female rats were exposed to NMP by gavage 5 days/week at 150, 450 or 1000 mg/kg/day 2 weeks before mating, during mating, gestation, and lactation.

The NOAEL of 125 g/kg bw/d (Saillenfait et al., 2002) is only slightly below the LOAEL of 150 mg/kg bw/d for maternal and developmental effects in the study of Sitarek et al. (2012). David et al. (2021) applied an assessment factor (AF) of 3 to the NOAEL of 125 mg/kg bw/d derived from the Saillenfait et al., (2002) study to consider the uncertainties in the underlying database. Further Afs - 10 of each - were applied to account for inter- and intraspecies differences, resulting in a TRV-like value (toxicity reference value) of 0.42 mg/kg bw/d. The TRV-like value is a value for external exposure guidance which is calculated according to the generally accepted rules provided by ECHA (ECHA, 2012; Chapter R.8).

By using a mass balance equation and assuming steady state conditions, an HBM-GV_{GenPop} of 15 mg/L for adolescents and adults (rounded value) and 10 mg/L for children (rounded value) was calculated for the sum of the selected urinary exposure biomarkers 5-HNMP and 2-HMSI (David et al., 2021).

3.3.2.2 Derivation of HBM-GVGenPop for NEP

NEP is classified as toxic for reproduction Cat. 1B – H360D acc. to CLP regulation based on the developmental studies on NEP showing an increase in postimplantational losses, the reduction of body weight of foetuses and the induction of malformations.

- Choice of biomarkers of exposure for NEP

As substance-specific biomarkers for NEP exposure the two metabolites 5-HNEP (5-hydroxy-N-ethyl-2-pyrrolidone) and 2-HESI (2-hydroxy-N-ethylsuccinimide) are recommended to be analysed in urine (Koch et al., 2014).

- Choice of the key study and the application of assessment factors

The current knowledge based on human exposure to NEP on whether and how it might cause human health effects is not sufficient to directly derive HBM-GVs based on human data. Thus, HBM-GVs are based on a point of departure (POD) identified in a key animal study. For NEP the dermal route of exposure is assumed to be the most relevant for the general population followed by inhalation and oral exposure.

For the reasons already given for the NMP, animal studies with oral exposure were considered relevant for the derivation of the HBM-GVs. Additionally, findings from “head-nose only” inhalation studies documented in the registrant summaries in the registration dossier from ECHA were considered (ECHA registration dossier, 2013).

Two developmental studies were considered relevant for the derivation of HBM-GV_{GenPop} values for NEP. The selected key study is the developmental toxicity study by Saillenfait et al. (2007) in rats with a LOAEL of 250 mg/kg bw/d (decrease in fetal body weight) and a NOAEL of 50 mg/kg bw/d.

A developmental study in rabbits with a LOAEL of 200 mg/kg bw/d and a NOAEL of 60 mg/kg bw/d (BASF, 2007) was also considered. Application of inter- and intraspecies assessment factors (10 for each) resulted in a TRV-like value of 0.5 mg/kg bw/d.

David et al (2021) compared this outcome with the inhalation route of exposure. The authors found that the database was rather limited and only allows a rough estimation of the body dose having no effects. A summary of a “head-nose only” subchronic study on rats is available in the registration dossier from ECHA (ECHA registration dossier, 2013). At the highest applied vapour concentration of 200 mg/m³ local effects on the mucous membranes of the nose were observed, but no systemic effects were apparent. Thus, this highest dose is regarded as NOAEC for systemic effects. David et al (2021) extrapolated this value regarding study duration and exposure conditions (200 mg/m³: 2 x 6/24 x 5/7 = 17.86 mg/m³) and also regarding to body dose per 24h (17.86 mg/m³ x 1.15 =

20.5 mg/kg bw/d (ECHA, 2012)). This would lead to a lower dose compared to the NOAEL of the key oral developmental study of 50 mg/kg bw/d. David et al. (2021) argue that it should be kept in mind that higher concentrations have not been tested so far and no information on a LOAEC for systemic effects via the inhalation path is available.

By using the mass balance equation and assuming steady-state conditions, an HBM-GV_{GenPop} of 15 mg/L for adolescents and adults and 10 mg/L for children was calculated for the sum of the selected urinary exposure biomarkers 5-HNEP and 2-HESI.

HBM-GVs for DMF and DMAC were derived for workers within HBM4EU. For the rationale behind the derivation of these values see Task 5.2 Deliverable Report D 5.9, part 5 for the DMF and part 6 for the DMAC.

In the present document the derivation of the HBM-GV_{Worker} for DMF is merely briefly described followed by more detailed description how an adjustment of this value is made to be applied for the general population. Please note that this was done merely for the purposes of comparison within the current risk assessment!

3.3.2.3 Derivation of HBM-GVWorker for DMF

DMF is readily absorbed vial all routes of exposure in humans.

DMF is classified as toxic for reproduction Cat. 1B – H360D acc. to CLP regulation (EC Regulation No 1272/2008). It may damage the unborn child, is harmful in contact with skin, causes series eye irritation and is harmful if inhaled. The liver is the main target for toxicity after acute exposure both for animals and humans.

- Choice of biomarkers of exposure for DMF

The main metabolites of DMF are N-methylformamide (NMF), N-hydroxymethylformamide (HMMF) and N-aceyl-S-(acetamidomethyl)-L-cysteine (AMCC). N-methylformamyde and N-hydroxymethylformarmide are also measured as total NMF (tNMF).

Total NMF (tNMF) and AMCC in urine are recommended by many countries for the biological monitoring of occupational exposure towards DMF. Since these are the most studied biomarkers to assess DMF exposure and related health effects of DMF at workplace, they were chosen for derivation of HBM-$GV_{Workers}$ for DMF.

- Choice of the key study and the application of assessment factors

Since data on the relationships between health effects and biomonitoring data are available for DMF, HBM-$GV_{Workers}$ can be derived on the basis of relevant human data.

Effects on liver function defined by abnormal increase of hepatic enzymes, e.g. ALT, AST and yGT were selected as the critical effect to derive HBM-$GV_{Workers}$.

tNMF in urine

The database of studies on tNMF measurements and related hepatic effects is large. For groups without hepatic effects, mean values for urinary tNMF concentration were between 7.75 and 22.3 mg/L (i.e. 6.7 and 50 mg/g creatinine). For groups with observed hepatic effects, the concentrations were between 13.6 and 14.9 mg/L (9.1 and 13.4 mg/g creatinine). For people with excessive alcoholic beverage consumption, hepatic effects were noted at lower exposures (tNMF urinary concentration of 4.5 mg/L).

Based on these data an HBM-$GV_{workers}$ of 10 mg/L urine or 10 mg/g creatinine has been derived by Lamkarkach and Messlin (2021).

AMCC in urine

Only a few studies exist examining the relationship between urinary concentrations of AMCC and hepatic effects.

No effects on liver enzymes were observed in a study from Germany (Kilo et al., 2016) at a mean AMCC concentration of 9.42 ±10.42 mg/g creatinine. A $BMDL_{10}$ of 155 mg/L (119 mg/L in male workers) was calculated for a group of workers with an increased incidence of liver injury in a study from China (Wu et al., 2017).

Lamkarkach and Meslin (2021) (Deliverable Report D5.9, part 5) stated that due to the paucity of data it was uncertain whether the recommendation of an HBM-$GV_{Workesr}$ for urinary AMCC would be appropriate. Based on the study from Germany (Kilo et al., 2016) a provisional HGM-$GV_{workers}$ of 10 mg/g creatinine was derived.

This provisional HBM-$GV_{Workers}$ was adjusted for application to the general population as follows:

10 mg/g creatinine was divided by an assessment factor of 10 to consider more sensitive subgroups of the population yielding a provisional HBM-GV_{GenPop} of 1 mg/g creatinine for the DMF metabolite AMCC.

Despite hepatic effects being the most sensitive effects, DMF is classified as being reprotoxic – Cat. 1B, H360D. Therefore, this effect must be considered in the workplace. In 2019 ECHA derived a DNEL for inhalation, which, based on current data, protects both from adverse liver effects and also from developmental effects. The DNEL of 6 mg/m³ for both effects is proposed.

Note:

Since no exposure data for the general population in Europe for DMAC are available, the derivation of the respective HMB-GV in question is not outlined in this risk assessment.

3.4 Exposure assessment

3.4.1 Production volume of NMP, NEP, DMAC and DMF, and potential uses

The tonnages annually manufactured and/or imported in the European economic area of NMP, NEP, DMAC and DMF are given in Table 3 (acc. to information provided in the ECHA website).

Table 3: Annually manufactured and/or imported tonnages in the European economic area of NMP, NEP, DMAC and DMF (source: ECHA website)

Substance	Annual Production Volume [t/year]
NMP	10,000 – 100,000
NEP	1,000 – 10,000
DMAC	10,000 – 100,000
DMF	10,000 – 100,000

Unfortunately, no country-related information is available or can be identified.

Potential uses of NMP

Applications in professional settings and industry are manifold (German HBM Commission, 2015a). As summarised by David et al. (2021), NMP is used as a solvent e.g. for the extraction of aliphatic

and aromatic hydrocarbons in the petrochemical industry, in the production of polymers (membranes), in coating products and waterborne prints. A further use is the application in cleaning agents for the removal of paints and coatings and also in the microelectronics fabrication industry in stripping and cleaning applications. In addition, NMP is used both in lithium ion batteries and in other hybrid batteries, in functional fluids such as coolants. Applications are also described for the pharmaceutical industry.

Consumer products potentially including NMP are inks (for printers), toners, coatings, cleaners (RIVM, 2013a (Annex XV Restriction Report)).

Potential uses of NEP

NEP has been introduced in many applications as a substitute for NMP, e.g. in coatings and in cleaning agents (German HBM Commission, 2015b). In industry NEP is used as a solvent, catalyst and cationic surfactant.

NEP is used in anti-freeze products, coating products, lubricants and greases, adhesives and sealants, care products, non-metal-surface treatment products, inks and toners, in leather treatment products, polishes, waxes and cleaning products (acc. to ECHA information).

Potential uses of DMAC and DMF

For DMAC and DMF similar uses are plausible. The main sectors highlighted by ECHA are industrial uses for laboratory chemicals, in the manufacturing of other substances (use as intermediates) and in the production of other chemicals and plastic products.

More detailed information on potential uses and potential releases to the environment can be found in the ECHA website.

3.4.2 Human exposure

Human exposure routes

Indoor air, relevant product use and manufacturing are the main sources of exposure for the all four aprotic solvents (acc. to information provided in the ECHA website).

Particularly for NMP and NEP, the exposure routes are inhalation, dermal uptake and oral ingestion.

Availability of Human Biomonitoring data in general

A literature search revealed the scarcity of Human Biomonitoring data with respect to the aprotic solvents in question for the general population. Tables A 6 – A 9 given in the Annex summarise the results of the literature search done.

A single studies have been carried out on NMP and NEP.

For DMAC Human Biomonitoring data are available for the occupational population only (for an overview see the Table A8 in the Annex).

Some background data for DMF in relation to unexposed workers are available in an occupational study by Kilo et al., 2016. In addition, 360 samples from the German Environmental Specimen Bank have been analysed recently specifically within the HBM4EU to detect the level of metabolite AMCC in the general population (more information below).

3.4.2.1 General population: NMP and NEP

For NMP and NEP Human Biomonitoring data are available from the German Environmental Specimen Bank (ESB) (Ulrich et al., 2018) and the German Environmental Survey of Children and

Adolescents V (GerES V) (Schmied-Tobies et al., 2021). The key information on the two studies is given in the Table 4.

Table 4: Key information on HBM studies in the general population

Study	Sample n	Study population	Biomarkers investigated	Time span	Sample type	Additional investigations
ESB (Ulrich et al., 2018)	540	students, aged 20-30, area of Münster (Germany); 60 per sampling year (30 male, 30 female)	5-HNMP 2-HMSI 5-HNEP 2-HESI	1991, - 2014	24-h urine samples	time trends of exposure/influence of regulatory measures
GerES V (Schmied-Tobies et al., 2021)	2178	children and adolescents, German residents, ages 3-17	5-HNMP 2-HMSI 5-HNEP 2-HESI	2014-2017	morning urine	association of exposure with a variety of factors; use of questionnaires to collect information on habits and behaviours

ESB = German Environmental Specimen Bank; GerES V = German Environmental Survey of Children and Adolescents V

The findings of the two publications in question are presented and discussed in the following part of this document below. In the Table 5 some key data of the two studies are summarised.

Table 5: Urinary alkyl pyrrolidone metabolite concentrations of participants from the German Environmental Specimen Bank (ESB[a]) from the years 1991 – 2014 and of participants from the German Environmental Survey of Children and Adolescents (GerES V[b]) from the years 2014 - 2017

Volume-based conc.	N	%≥ LOQ	GM, µg/L	P50 (Median), µg/L	P95, µg/L	Max, µg/L	Reference/study
∑NMP metabolites	540	-	-	-	-	1013	ESB[a]
	2178	-	103.1	104	283	1610	GerES V[b]
5-HNMP	540	98	29.1	30.3	98.1	655	ESB[a]
	2178	100	56.01	56.2	183	1130	GerES V[b]
2-HMSI	540	99.6	38.0	38.8	100	358	ESB[a]
	2213	100	45.08	45.0	106	523	GerES V[b]
∑NEP metabolites	540	-	-	-	-	1312	ESB[a]
	2164	-	11.86	7.5	315	3140	GerES V[b]
5-HNEP	540	34.8	2.8	<LOQ	212	962	ESB[a]
	2199	32	3.06	<LOQ	144	1880	GerES V[b]
2-HESI	540	75.7	8.8	6.1	230	950	ESB[a]
	2179	87	7.57	5.5	152	1340	GerES V[b]

[a]Ulrich et al., 2018; [b]Schmied-Tobies et al., 2021

N = number of samples tested; GM = geometric mean; P50, P95 = percentile; MAX = maximum value, LOQ = Limit of quantification; % ≥ LOQ = percentage of samples showing concentration equal to LOQ or exceeding it

The NMP metabolites 5-HNEP and 2-HMSI could be quantified in the vast majority of samples (Ulrich et al., 2018) or in all samples (Schmied-Tobies et al., 2021). In contrast, the NEP metabolites could be quantified to a lesser extent in both studies (Table 5). Both studies clearly show the ubiquitous exposure of the general population to NMP and NEP.

Also, the GM concentrations for the analysed metabolites of NMP and NEP were very similar in the both two studies covering ESB and GerES V, accordingly:

- For NMP: 5-HNMP (29.1 µg/L vs. 56.01 µg/L) and for 2-HMSI (38.0 µg/L vs. 45.08 µg/L);
- For NEP: 5-HNEP (2.8 µg/L vs. 3.06 µg/L) and for 2-HESI (8.8 µg/L vs. 7.57 µg/L)
- (Table 5).

Schmied-Tobies et al. (2021) reported GM concentrations for the sum of both NMP metabolites (∑NMP) of 103.1 µg/l and for the sum of both NEP metabolites (∑NEP) of 11.86 µg/L. The authors calculated daily intakes (DIs) based on the urinary metabolite levels. The GM of the resulting daily intakes was 2.085 µg/kg$_{bw}$/day for NMP and 0.309 µg/kg$_{bw}$/day for NEP (comparison of DIs of the two studies is reflected in the Table 6).

Table 6: Calculated daily intakes (DIs) for NMP and NEP

DIs	N	GM, µg/kg$_{bw}$/day	P50 (Median), µg/kg$_{bw}$/day	P95, µg/kg$_{bw}$/day	Max, µg/kg$_{bw}$/day	Reference/study
NMP	-	-	2.2 (all years)	5.5 (all years)	-	ESB[a]
	2176	2.085	2.06	5.22	22.6	GerES V[b]
NEP	-	-	0.3 (all years)	20.1 (all years)	-	ESB[a]
	2161	0.309	0.20	5.76	74.6	GerES V[b]

[a]Ulrich et al., 2018; [b]Schmied-Tobies et al., 2021; DIs = daily intakes

3.4.2.1.1 Time trend of exposure to NMP and NEP and effect of regulatory measures

The samples from the environmental specimen bank (ESB) (Ulrich et al., 2018) were taken from 1991-2014, thus allowing the investigation of time trends. Over the investigated time span, the authors observed variations of concentrations within rather tight boundaries (Table 7 and Figure 1). Surprisingly, this was even observed for NEP which – as a substitute for NMP - has been introduced only in the last decade. The authors observed a slight increase in the DIs of NMP over time ($p<0.001$), whereas no trend was seen for NEP ($p<0.080$).

Since NEP is restricted under Annex XVII of REACH in 2014[1] and the similar regulatory measures for NMP came into force in 2018 with transitional period[2] as well as both NMP and NEP are listed in Annex II of the Cosmetic Products Regulation No1223/2009 since 2020[3] only, further monitoring is suggested to investigate the effectiveness of these measures.

[1]With restriction wording "shall not be placed on the market as substances, constituents of other substances or components of a mixture above 0.3 %" (https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32014R0317)
[2]With restriction wording "shall not be placed on the market as substances, constituents of other substances or components of a mixture above 0.3 %"; in force after 9 May 2020 unless specific conditions are not met (https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32018R1513)
[3]List of substances prohibited in cosmetic products (https://data.europa.eu/data/datasets/cosmetic-ingredient-database-2-list-of-substances-prohibited-in-cosmetic-products?locale=en)

Table 7: Median and 95th percentile of the NMP and NEP metabolite concentrations for the investigated years (source: Ulrich et al., 2018 supplemental material: SI 3)

Concen-tration	NMP				NEP			
	5-HNMP, µg/L		2-HMSI, µg/L		5-HNEP, µg/L		2-HESI, µg/L	
year	median	95th percentile	median	95th percentile	median	95th percentile	median	95th percentile
1991	25.9	79.5	40.0	95.6	<LOQ	337	22.5	300
1995	27.8	92.2	45.8	121	<LOQ	194	6.7	282
1999	33.1	95.6	38.3	95.0	9.7	237	38.4	314
2003	26.5	83.4	39.1	101	<LOQ	248	8.5	182
2006	30.2	95.0	42.4	84.7	<LOQ	111	5.2	197
2008	27.6	79.0	35.8	98.4	<LOQ	70.0	2.5	70.7
2010	42.1	154	36.9	104	<LOQ	116	2.8	155
2012	26.2	62.4	31.4	73.5	<LOQ	133	4.0	215
2014	37.6	112	39.0	111	<LOQ	217	15.7	167

LOQ = Limit of quantification

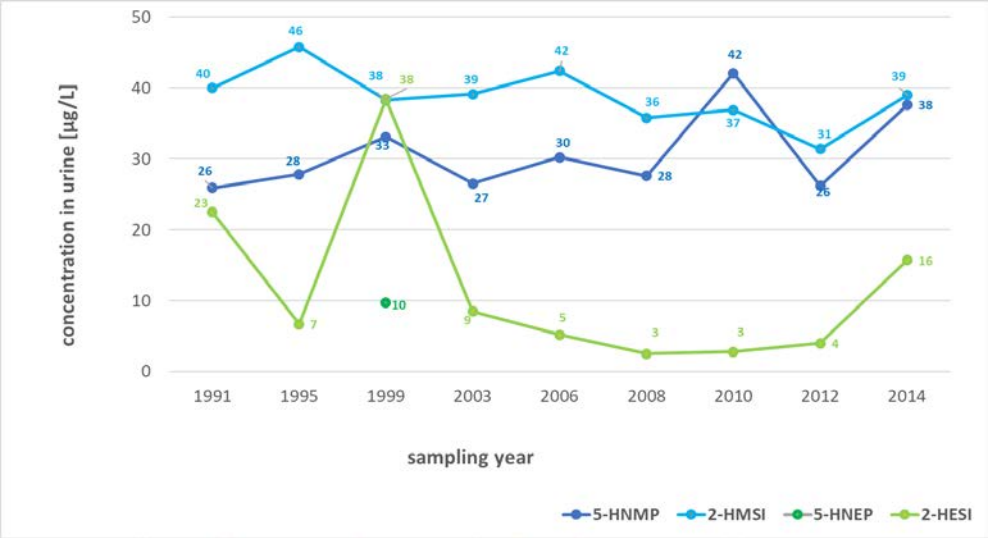


Figure 1: P50 concentration of the NMP and NEP metabolites 5-HNMP, 2-HMSi, 5-NEP and 2-HESI for the investigated years (data from Ulrich et al., 2018, supplemental material SI 3)

Note: The NEP metabolite`s 5-HNEP data are only available for 1999, in all other years the P50 concentrations were below Limit of detection (<LOD) and Limit of quantification (<LOQ).

3.4.2.1.2 Daily intakes (DIs) for NMP and NEP for various subgroups

Schmied-Tobies et al. (2021) calculated the DIs of NMP and NEP for various subgroups. These included related age group, community size, socioeconomic status, migration background, textile flooring in home, usage of oven cleaners, usage of shampoo etc. Clear age-related differences could be found. For NMP, a decrease with increasing age can be observed. For NMP children aged 3-5 years had a 55% higher intake compared to adolescents aged 14-17 years, but for NEP a 32% lower intake has been observed (comparisons based on GMs of DIs). Children and adolescents living in larger communities (≥100.000 inhabitants) had a higher DI for NMP than those living in medium-sized communities. Low socioeconomic status was associated with a DI

that was twice as high as for a high socioeconomic status for NEP, but this was not observed for NMP. Finally, the migration background of the children and adolescents was found to be significantly associated with exposure to both NMP and NEP. Participants with a two-sided migration background[4] had a 13% higher DI of NMP compared to those with no migration background. In addition, the DI of NEP was even twice as high for this group of participants.

3.4.2.1.3 Potential sources of exposure

In GerES V (Schmied-Tobies et al., 2021) the application of a variety of products either containing or manufactured by using alkyl pyrrolidones was surveyed. The authors compared the DIs of participants who had been in contact (according to the questionnaire) with these products with the DIs of participants who had minor or no exposure.

For children and adolescents exposed to floor cleaners, oven cleaners or fabric softeners the GM of the DI of NEP was found to be elevated by 15% to 32% compared to individuals not exposed to these products. Schmied-Tobies et al. (2021) found no associations regarding textile flooring, usage of graffiti remover, disinfectants or furniture polish. For participants reporting a frequent usage of body wash/shower gel or shampoos a 53% to 73% higher exposure was found compared to those never using these products. Since both NMP and NEP have been prohibited in cosmetics since 2020 (EU, 2019), this association is not expected in the future. Regarding the usage of body lotions, cremes or nail polish no association was found. The usage of facial or eye make-up showed no clear association with NEP exposure.

The authors conclude that inhalation exposure e.g. from textile flooring might be of minor importance compared to dermal absorption of cosmetics and personal care products.

3.4.2.2 General population: DMAC and DMF

The literature search revealed that there are currently no HBM data for the general population for DMAC. Therefore, Human Biomonitoring of this substance is highly recommended in Europe.

For DMF some data are available from a study on occupational exposure by Kilo et al. (2016) where exposed and non-exposed workers were analysed for AMCC in urine.

Within HBM4EU samples from the German Environmental Specimen Bank (ESB) were analysed for the DMF metabolite AMCC in a total of 360 samples. Key information on the two studies in question is given in the Table 8.

[4] „Migration backround“ is a special German statististical category. „One-*sided migration background“* means that only one parent has a *migration background and „Two-sided migration background“ that both parents have a migration background*

Table 8: Key information on HBM studies investigated

Study	Sample n	Study population	Biomarkers investigated	Time span	Sample type	Additional investigations
ESB (unpu-blished)	360	Students, aged 20-30, area of Münster (Germany); 60 per sampling year (30 male, 30 female)	AMCC	2000 - 2021	24-h urine samples	Time trends of exposure/influence of regulatory measures
Kilo et al. 2016	175	Adult workers, Average age 45	AMCC	2016	End-of-shift samples	Effects on liver enzymes

ESB = German Environmental Specimen Bank

Some key data of the two studies are presented In the Table 9. The data are given in mg/g creatine for comparability with data from Kilo et al. 2016. Data from Kilo et al. (2016) are somewhat higher than those from the German ESB.

Table 9: Urinary DMF (metabolite AMCC) concentrations in mg/g creatinine from Kilo et a. (2016) and the German ESB

Reference/study	Arithmetic mean	Standard deviation	P50	Min	Max
Kilo et al. 2016	0.28	0.21	0.21	0.004	1.16
ESB 2015	0.13	0.11	0.082	0.021	0.48
ESB 2021	0.099	0.090	0.067	0.018	0.405

ESB = German Environmental Specimen Bank

3.4.2.2.1 Time trend of exposure to DMF and effect of regulatory measures

The analysed samples from the German environmental specimen bank (ESB) were taken between 2000-2021, thus allowing the investigation of time trends (unpublished data). Over the investigated time span a decrease in concentrations of > 50% can be observed (Table 10 and Figure 2). As the DMF is regulated in the Cosmetic Products Regulation No1223/2009 since 2010[5], the observed decrease is assumed to be partly due to the regulatory measures being effective.

In addition, DMF is restricted under Annex XVII of REACH in 2018 with transitional period till 2020, not giving possibility to make firm conclusions on the effectiveness of these regulatory measures up to now, however, some influence on the reduction trend could be assumed.

[5] List of substances prohibited in cosmetic products (https://data.europa.eu/data/datasets/cosmetic-ingredient-database-2-list-of-substances-prohibited-in-cosmetic-products?locale=en)

Table 10: DMF (metabolite AMCC) concentrations for the investigated years (source: German ESB, unpublished data)

Year	N	% >LOQ	MIN, µg/L	GM, µg/L	CI low, µg/L	CI high, µg/L	P50, µg/L	P95, µg/L	MAX, µg/L
2000	60	100	13,0	98,7	83,7	116,5	103,5	294,7	523,0
2005	60	100	20,9	68,7	57,8	81,8	68,9	214,4	305,0
2010	60	100	20,5	76,8	65,5	90,0	67,5	189,1	651,0
2015	60	100	12,9	60,9	50,2	73,8	58,4	210,7	350,0
2019	60	100	8,1	56,8	45,4	71,2	54,9	231,9	388,0
2021	60	100	7,3	45,6	36,6	56,8	48,4	157,5	225,0

N = number of samples tested; MIN = minimum value; GM = geometric mean; CI low and CI high = range of 95 confidence interval; P50, P95 = percentile; MAX = maximum value; LOQ = limit of quantification; % ≥LOQ = percentage of samples showing concentration equal to LOQ or exceeding it.

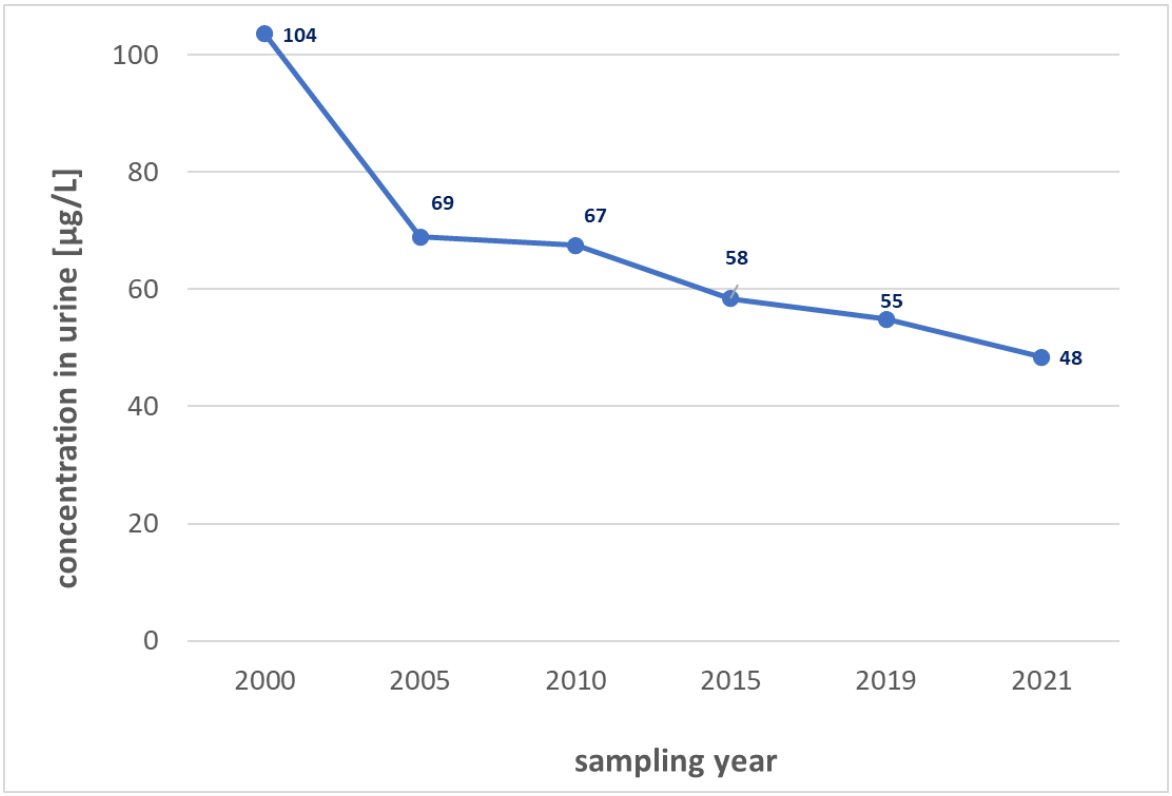


Figure 2: P50 concentration of the DMF metabolite AMCC for the investigated years (German ESB, unpublished data)

3.5 Risk characterisation and uncertainty analysis

3.5.1 Exposure to NMP and NEP: Comparison with Human Biomonitoring Guidance Values (HBM-GVs)6

Health-related Human Biomonitoring Guidance Values (HBM-GVs) are intended for direct comparison with measured values (for details see Apel et al., 2020).

The exposure data (P95 and maximum values) from the two studies from Germany (i.e., Ulrich et al., 2018 and Schmied-Tobies et al., 2021) are compared with the newly derived HBM-GV_{GenPop} both for NMP and NEP. Thereby three age groups are considered: children (3-13 years), adolescents (14-17 years) and young adults (20-30 years).

For this purpose, the GerES data have been reprocessed to enable this comparison.

The maximum concentration found by Schmied-Tobies et al. (2021) for children up to 13 years, when analysing data from GerES V for ∑NMP metabolites, was 1600 µg/L (for the age group: 11-13 years). This is a factor of 6 below the newly derived HBM-GV_{GenPop} of 10 mg/L for children The maximum concentration for ∑NEP metabolites for children was 1520 µg/L (for the age group: 6-10 years) which is a factor of 7 below the HBM-GV_{GenPop} of 10 mg/L for children. The maximum concentration found by Schmied-Tobies et al., (2021) for adolescents (14-17 years) was 1610 µg/L for the ∑NMP metabolites, which is a factor of 9 below the newly derived HBM-GV_{GenPop} of 15 mg/L for adolescents and adults. For ∑NEP metabolites the maximum value found for adolescents was 3140 µg/L which is a factor of 5 below the newly derived HBM-GV_{GenPop} for adolescents and adults.

The maximum concentration found by Ulrich et al. (2018) by analysing data from the ESB for ∑NMP metabolites was 1013 µg/L. This is a factor of 15 below the newly derived HBM-GV_{GenPop} of 15 mg/L for adolescents and adults. The maximum concentration for ∑NEP metabolites was 1912 µg/L which is a factor of 8 below the HBM-GV_{GenPop} of 15 mg/L for adolescents and adults.

Comparison of the P95 and maximum values from the two studies for ∑NMP metabolites (Figure 3) and for ∑NEP metabolites (Figure 4) for the three age groups - children, adolescents and young adults - is presented below.

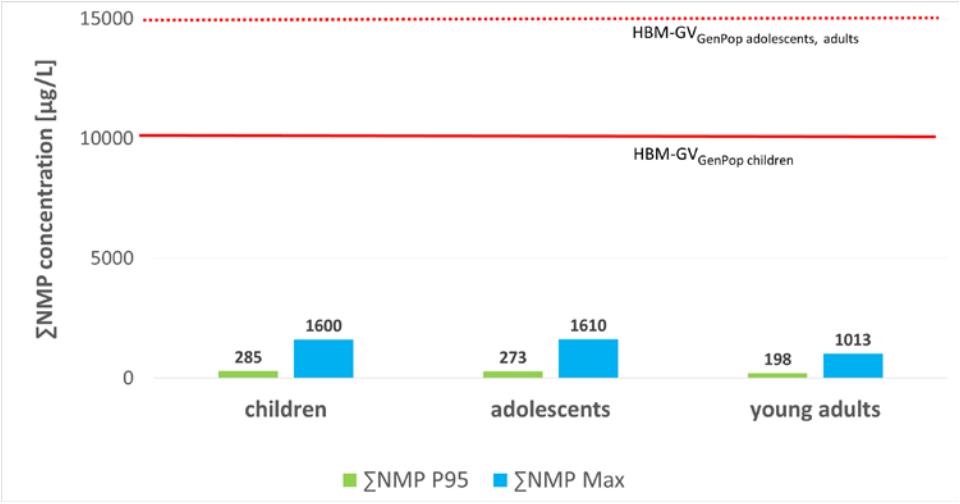


Figure 3: Comparison of exposure data for NMP from GerES V (Schmied-Tobies et al., 2021) and ESB (Ulrich et al., 2018) with the newly derived HBM-GV_{GenPop} for children, adolescents and young adults based on P95 and maximum values

[6] this is meanwhile published (David et al., 2021); parts of the text are therefore identical with the corresponding paragraphs

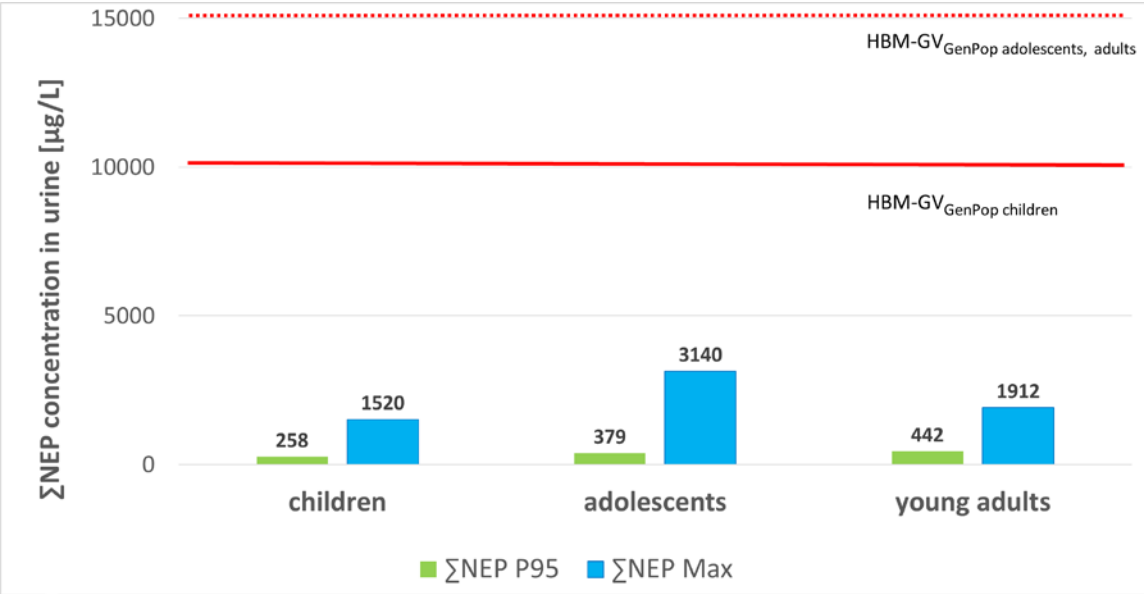


Figure 4: Comparison of exposure data for NEP from GerES V (Schmied-Tobies et al., 2021) and ESB (Ulrich et al., 2018) with the newly derived HBM-GV_{GenPop} for children, adolescents and young adults based on P95 and maximum values

3.5.2 Exposure to NMP and NEP: considering combined exposure: The hazard index (HI) approach

Since it has to be assumed that in real life a concurrent exposure to NMP and NEP can occur and both substances have similar toxicological profiles the mixture effects of the two substances are to be considered (German HBM Commission, 2015a). In order to assess the cumulative risk of NMP and NEP the hazard index (HI) was calculated as the sum of the hazard quotients (HQs) for NMP and NEP, where HQ is the ratio between the sum of 2-HMSI and 5-HNMP concentrations (for NMP) and 2-HESI and 5-HNEP concentrations (for NEP), respectively, and the corresponding newly derived HBM-GV value. A hazard index < 1 would indicate that the exposure is below the newly derived HBM-GV considering combined exposure to NMP and NEP:

$HI = HQ_{NMP} + HQ_{NEP}$

HQ_{NMP} = [2-HMSI] + [5-HNMP] / HBM-GV

HQ_{NEP} = [2-HESI} + [5-HNEP] / HBM-GV

HI ≤1, no risk is anticipated

HI >1, at risk

In the Table 11 the resulting HIs are shown for both studies, comprising three age groups, i.e. children (4-10 years), adolescents (14-17 years) and young adults (20-30 years).

The hazard index based on the newly derived HBM-GVs for NMP and NEP considering co-exposure of these solvents was 0.3 at maximum [maximum HQs: 0.1 (NMP) and 0.2 (NEP)] using the data from Schmied-Tobies et al., 2021, both for children aged 3-13 and adolescents aged 14-17 years. The calculated HI using the data produced by Ulrich et al., 2018 are similar. The maximum HI was 0.2 (maximum HQs: 0.07 for NMP and 0.1 for NEP] for the young adults).

Table 11: Hazard index considering combined exposure of NMP and NEP

Age group (years) / study	Children (3-13) / GerES V	Adolescents (14-17) / GerES V	Young adults (20-30) / ESB
HI (P95)	0.05	0.04	0.04
HI (max)	0.3	0.3	0.2
HI ≤ 1	✓	✓	✓

GerES V: Schmied-Tobies et al., 2021; ESB: Ulrich et al., 2018

As can be seen in the Table 11, all resulting HI values were below 1. According to current knowledge and based on isolated examination of the N-alkyl pyrrolidones under investigation, exposure of children, adolescents and young adults in Germany to NMP and NEP (alone or in combination with each other) does not give reason for toxicological concern. However, a possible combined exposure with other reprotoxic substances present in the environment should be considered in “real-life-situations”, since these might increase the risk for common effects (Kortenkamp and Faust, 2018). Kortenkamp (2020) reported on efforts that have already been undertaken to identify pathways converging at critical notal points to produce down-stream adverse effects (so-called adverse outcome pathways (AOPs)).

3.5.3 DMAC and DMF: comparison with Human Biomonitoring Guidance Values (HBM-GVs)

Since no HBM data are available for DMAC for the general population, the related risk assessment cannot be performed at the moment.

In order to be able to assess the contribution of DMF to the total exposure of the aprotic solvents that are being investigated here, a risk assessment was performed for DMF using a provisional HBM-GV_{GenPop} derived only for this purpose.

The P95 and maximum value measured in urinary samples from the German ESB for the DMF metabolite AMCC in relation to young adults are compared with the provisional HBM-GV_{GenPop} for DMF.

The provisional HBM-GV_{GenPop} for DMF is a factor of 2.5 higher than the maximum value measured in samples from the ESB.

In a second step the combined exposure to NMP, NEP and DMF is assessed for young adults using the hazard index approach. The results are given in the Table 12.

Table 12: Hazard index: considering combined exposure of NMP, NEP and DMF for young adults

HI	Young adults (20-30 years)
HI (P50)	0,07
HI (P95)	0,34
HI (Max)	0,60
HI ≤ 1	✔

As can be seen in Table 12, all resulting HI values were well below 1, also when including DMF in the assessment.

3.5.4 Uncertainty analysis

3.5.4.1 Exposure assessment

The risks are rather underestimated than overestimated because the real life exposure might be underestimated due to exposure to other reprotoxic compounds. Other reprotoxic chemicals that are present could add to the toxicity of the aprotic solvents under investigation and thus, contribute to the total risk. It can be recommended that mixture risk assessment needs to be considered (see above).

The study population is representative for the German population aged 3-17 (Schmied-Tobies et al., 2021), whereas only students (aged 20-30) were investigated in Ulrich et al. (2018) study. A comparison of the two studies is limited by the differing study design, difference in the populations that were investigated, difference in sampling (24 h urine samples vs. first-morning void urine samples) and due to different study years.

Furthermore, as data were found only reflecting the exposure situation in Germany, for an assessment of the exposure of the whole European population, representative HBM data are needed from the other countries within Europe covering all age groups.

3.5.4.2 Derivation of Human Biomonitoring Guidance Values (HBM-GVs)

In the strategy paper for derivation of HBM-GVs (Apel et al., 2020) it is suggested to indicate a level of confidence (i.e., low, medium or high values) for each calculated HBM-GV. This level of confidence (LoC) should reflect the uncertainties identified during the derivation of the HBM-GV. Since the derivation of HBM-GVs is based on very conservative scenarios and default assumptions (Apel et al., 2020) this does not necessarily mean a low level of protection. The level of confidence takes into account the various uncertainties underlying the derivation of the HBM-GV (like reliability of the key study used to derive the TRV-like value or uncertainties related to the toxicokinetic data on the substance of interest).

For NMP the overall LoC was set to “medium”, for NEP the LoC was set to “medium/low”. For both substances, the information on toxicity is very limited and the assessment of toxicity is based mainly on animal studies. For more details see Deliverable report D 5.9.

For DMF the overall LoC attributed to the HBM-$GV_{workers}$ are set to “high for tNMF and “medium-low” for AMCC. For AMCC available studies are limited. The results concerning levels of AMCC linked to hepatic effects reported in occupational studies are not consistent.

3.6 Discussion and conclusions

3.6.1 General discussion and conclusions of the work performed

3.6.1.1 Results in the light of policy questions

3.6.1.1.1 Building a picture of internal exposure burden for NMP and NEP (eventually DMAC and DMF) within Europe based on available HBM data

The results of the literature search clearly indicate a data gap for exposure in the whole of Europe towards the investigated aprotic solvents (i.e., NMP, NEP, DMAC and DMF). A picture of internal exposure burden could only be attained for NMP and NEP for the German population (aged 3-17) and students in Germany (aged 20-30) as well as for DMF for students in Germany (aged 20-30).

3.6.1.1.2 Gaining more knowledge on the most vulnerable and highly exposed population groups

Due to the scarcity of available data, the knowledge gain is restricted to the German population and the age groups 3-17 and 20-30 (with further restrictions since only students were investigated).

For NMP, the daily intake (DI) decreased with increasing age, 3–5-year-old children had a 55% higher intake of NMP than adolescents aged 14–17 years (Schmied-Tobies et al., 2021).

For NEP, the daily intake (DI) increased with increasing age. Here, 3–5-year-old children had a 32% lower intake of NEP compared to adolescents aged 14–17 years (Schmied-Tobies et al., 2021).

Children and adolescents living in larger communities (≥100.000 inhabitants) had a higher DI of NMP compared to those living in medium-sized communities (Schmied-Tobies et al., 2021).

For NEP, low socioeconomic status was associated with a DI that was twice as high as for high socioeconomic status. This was not observed for NMP (Schmied-Tobies et al., 2021).

Furthermore, the migration background of the participants was found to be significantly associated with exposure to both NMP and NEP. Participants with a two-sided migration background had a 13% higher DI of NMP compared to those with no migration background. The DI of NEP was twice as high for this group of participants (Schmied-Tobies et al., 2021).

3.6.1.1.3 Assessing if internal exposure is exceeding available HBM Guidance Values for the aprotic solvents

For the investigated population (German residents aged 3-17 and 20-30 years), internal exposures with NMP and NEP did not exceed the corresponding HBM-GV_{GenPop}. Even when considering a combined exposure of NMP and NEP the exposure stayed well below the Guidance Values. Nevertheless, it should be kept in mind that people are exposed to a variety of substances which might add to the toxicity of the investigated compounds.

For DMF data from young adults (20-30 years) from the German ESB were well below the provisional HBM-GV_{GenPop} derived for this assessment specifically.

3.6.1.1.4 Evaluating correlations of internal exposure with lifestyle behaviours, usage of certain products etc. based on German HBM data

For children and adolescents exposed to floor cleaners, oven cleaners, or fabric softeners, the GM of the DI of NEP was found to be elevated by 15% to 32% compared to individuals not exposed to these products (Schmied-Tobies et al., 2021).

For participants reporting on frequent usage of body wash/shower gel or shampoo, a 53% to 73% higher exposure was found compared to those never using these products (Schmied-Tobies et al.,

2021). Since both NMP and NEP are prohibited in cosmetics since 2020 (EU, 2019), this association is not expected to be in the future.

3.6.1.1.5 Evaluating risks of combined exposure to the reprotoxic aprotic solvents

When evaluating the risks of combined exposure to NMP and NEP it was found that the exposure data presented in the two studies from Germany (i.e., Ulrich et al., 2018 and Schmied-Tobies et al., 2021) stayed well below the newly derived HBM-GV_{GenPop}, for children, adolescents and adults.

For young adults it was possible to assess the combined exposure to NMP, NEP and DMF. It was found that the exposure data from the German ESB stayed well below the HBM-GV_{GenPop}.

Nevertheless, it should be kept in mind that people are exposed to a variety of substances which might add to the toxicity of the investigated compounds.

3.6.1.2 Recommendations for the regulatory risk assessment

With respect to DMF, the data recently obtained particularly within the HBM4EU by analysis of samples from the German environmental specimen bank (ESB) covering the time span 2000-2021 are showing effectiveness of regulatory measures (e.g. prohibition) being in place for cosmetic products since 2010. Over the investigated time span a decrease in concentrations of > 50% can be observed (Table 10 and Figure 2 above). In addition, restriction of DMF under Annex XVII of REACH in 2018 (however, with transitional period till 2020) could play some role as well.

As regards NMP and NEP, the HBM data available for 1991-2014 (ESB) and 2014-2017 (German Environmental Survey of Children and Adolescents V), cannot provide enough evidence for effectiveness of regulatory measures since NEP is restricted under Annex XVII of REACH in 2014 and NMP – since 2018 with transitional period till 2020. The same statement is true for prohibition of NMP and NEP in cosmetic products in EU as well being in force since 2020.

To sum up, further HMB investigations are needed to show effectiveness of regulatory measures being already in force and possible necessity for additional measures concerning NMP, NEP, DMAC and DMF exposure in a broader context covering the whole Europe.

3.6.1.3 Future prospects

The literature search revealed the scarcity of exposure data for the European general population for the four aprotic solvents (i.e., NMP, NEP, DMAC and DMF) in question.

Monitoring for these substances in the European population is therefore recommended in the future.

Further study populations should be investigated to broaden the database on exposure to the four aprotic solvents, including susceptible subpopulations such as pregnant women.

The sources of the aprotic solvents need to be further investigated and linked to environmental monitoring in different compartments as well as to indoor air monitoring in dwellings.

Only filling of the knowledge gaps indicated above can provide answers to policy questions formulated in the Scoping document for aprotic solvents in question.

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3.8 Annex

3.8.1 Abbreviations

2-HESI	2-hydroxy-N-ethylsuccinimide
2-HMSI	2-hydroxy-N-methylsuccinimide
5-HNEP	5-hydroxy-N-ethyl-2-pyrrolidone
5-HNMP	5-hydroxy-N-methyl-2-pyrrolidone
ACET	acetamide
ACGIH	American Conference of Governmental Industrial Hygienists
AF	assessment factor
ALT	Alanine amino peptidase
AMMA	S-(acetamidomethyl)mercapturic acid
AMCC	N-Acetyl-S-(N-methylcarbamoyl) cystein
AST	Aspartateamino peptidase
BAT	Biological Tolerance Values (Biologische Arbeitsstofftoleranzwerte)
BEI	Biological exposure index
BLV	Biological limit value
BMDL	Benchmark dose lower bound
BW	Body weight
CLP	Regulation on classification, labelling and packaging
DI	daily intake
DMAC	N,N-dimethylacetamide
DMF	N,N-dimethylformamide
DNEL	Derived no-effect level
ECHA	European Chemicals Agency
ESB	German Environmental Specimen Bank
GC-MS	Gas Chromatography-Mass Spectrometry
GerES V	German Environmental Survey of Children and Adolescents V
HBM-GV	HBM Guidance Value
$HBM\text{-}GV_{GenPop}$	HBM-GV for the general population
$HBM\text{-}GV_{Worker}$	HBM-GV for occupationally exposed adults
HBM	Human Biomonitoring
HBM4EU	European Human Biomonitoring Initiative
HI	hazard index
HPLC	high performance liquid chromatography,

HQ	hazard quotient
IARC	International Agency for Research on Cancer
γGT	Gamma glutamyl transpeptidase
GM	Geometric Mean
LOD	Limit of detection
LOQ	Limit of quantification
MAK	Maximum Workplace Concentration (Maximale Arbeitsplatz-Konzentration)
MCAMA	Methylcarbamoyl mercapturic acid
MCVal	N-methylcarbamoyl adduct at haemoglobin
MMAC	N-methylacetamide
NEP	1-ethylpyrrolidin-2-one
NMA	N-methylacetamide
NMHb	N-methylcarbamoylated haemoglobin
NMF	N-methylformamide
NMP	1-methylpyrrolidin-2-one
NO(A)EC	No Observed (Adverse) Effect Concentration
NO(A)EL	No Observed (Adverse) Effect Level
OEL	Occupational exposure limit
POD	Point of departure (Ausgangspunkt z.B. NOAEL für Risikoabschätzung)
RAC	Committee for Risk Assessment (REACH)
REACH	Registration, Evaluation, Authorisation and Restriction
RIVM	Netherlands National Institute for Public Health and the Environment
RfC	Reference concentration
SCCS	Scientific Committee on Consumer Safety
SCOEL	Scientific Committee on Occupational Exposure Limits
SEAC	Committee for Risk Assessment and Socio-Economic Analysis
tNMF	Total N-methylformamide
TLV-STEL	Threshold limit value – short-term exposure limit
TLV-TWA	Threshold limit value – time-weighted average
TLV	Threshold limit value
TRV	Toxicological Reference Value

3.8.2 Existing Regulatory values

Table A 9: Existing regulatory values for NMP

Biological Limit Values (occupational)		
value	**remarks**	**reference**
20 mg/g creatinine (2-HMSI)	urine, 18h post-shift	SCOEL, INCHT from
70 mg/g creatinine (5-HNMP)	urine, 2-4 h post-shift	SCOEL, INCHT from
100 mg/L (5-HNMP)		ACGIH https://www.acgih.org/
60 mg/g creatinine (5-HNMP) 75 mg/g creatinine (5-HNMP)	urine, 2-4 h post-shift, without workload urine, 2-4 h post-shift, workload (75 Watt)	Danish EPA 2014
	for additional OELs for EU and Non-EU countries please see	
HBM GVs (general population)		
value	**remarks**	**reference**
15 mg/L (adolescents, adults) 10 mg/L (children)	Sum of metabolites 5-HNMP and 2-HMSI; HBM GV_{GenPop}	David et al., 2021
10 mg/L HBM 1 (children) 30 mg/L HBM 2 (children) 15 mg/L HBM 1 (adults) 50 mg/L HBM 2 (adults)	Sum of metabolites 5-HNMP and 2-HMSI	HBM-Commission (2015a); Apel et al., 2017

Occupational Exposure Limits		
value	**remarks**	**reference**
40 mg/m^3 or 10 ppm (8h TWA) 80 mg/m^3 or 20 ppm (short term)		SCOEL REC 119
10 mg/m^3 (DNEL)	chronic inhalation exposure workers covering pregnant woman	ECHA RAC, NMP Restriction Proposal https://echa.europa.eu/documents/10162/aa77c7c4-4026-4ab1-b032-8a73b61ca8bd

Table A 2: Existing regulatory values for NEP

HBM GVs (general population)		
value	**remarks**	**reference**
10 mg/L HBM 1 (children) 25 mg/L HBM 2 (children) 15 mg/ L HBM 1 (adults) 40 mg/L HBM 2 (adults)	Sum of metabolites 5-HNEP and 2-HESI	HBM-Commission ; Apel et al., 2017
15 mg/L (adolescents, adults) 10 mg/L (children)	Sum of metabolites 5-HNEP and 2-HESI	HBM GV_{GenPop} (task 5.2 Deliverable Report D 5.9), David et al., 2021
10 mg/m^3 (DNEL)	chronic inhalation exposure workers covering pregnant woman	ECHA

Table A 3: Existing regulatory values for DMF

Biological Limit Values (occupational)		
value	**remarks**	**reference**
HBM GVWorker tNMF: 10 mg/L; 10 mg/g creatinine AMCC: 10 mg/g cr	AMCC: provisional HBM-GVWorker	Deliverable Report D 5.9. part 5
15 mg/L (NMF)	urine at the end of shift at the end of work week	SCOEL (2015)
30 mg/L (NMF)	urine at the end of shift at the end of work week	ACGIH https://www.acgih.org/
Occupational Exposure Limits		
value	**remarks**	**reference**
15 mg/m3 or 5 ppm (8h TWA) 30 mg/m3 or 10 ppm (short term)		OEL https://echa.europa.eu/de/substance-information/-/substanceinfo/100.000.617
30 ug/m3	Reference concentration	US EPA https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0511_summary.pdf

Table A 4: Existing regulatory values for DMAC

Biological Limit Values (occupational)		
value	**remarks**	**reference**
HBM GV_{Worker} 12 mg/g creatinine tNMAC		Deliverable Report D 5.9. part 6
20 mg/g creatinine (DMAC)	urine at the end of shift at the end of the work week	
30 mg/g creatinine (NMAC)	urine at the end of shift at the end of the work week	ACGIH https://www.acgih.org/
30 mg/g creatinine (NMAC)	urine at the end of shift at the end of the work week	DFG
100 mmol creatinine		UK biological monitoring guidance values
Occupational Exposure Limits		
value	**remarks**	**reference**
36 mg/m^3 or 10 ppm (8h TWA) 72 mg/m^3 or 20 ppm (short term)		SCOEL (2015)

Additionally, neither NHANES nor Health Canada does assess the four aprotic solvents in their biomonitoring surveys.

3.8.3 Experimental – toxicokinetic studies

Tab: A 5: Toxicokinetic studies NMP and NEP

Study type	Study design	Metabolites	Results	Analytical method	Reference
NMP					
Toxicokinetic study) dermal absorption)	6 female + 6 male volunteers (group 1 and 2) and additional group of 6 males (group 3) topically exposed for 6 h to 300 mg NMP or 300 mg NMP in 50% water solution; blood and urine sampling before, during and up to 9 d after exposure	5-HNMP 2-HMSI	max concentration of 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-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. Preferably 2-HMSI should be used as the biomarker of exposure to NMP	MS	
Toxicokinetic study	6 male volunteers exposed for 8 h to NMP in concentrations of 0, 10, 25 and 50 mg/m^3 blood and urine sampled before, during and up to 40 h after exposure	5-HNMP	Mean plasma concentration [P-(5-HNMP)] after 8-hour NMP exposure to 10, 25, and 50 mg/m3 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. Conclusions: 5-HNMP is an excellent biomarker for assessing exposure to NMP	GC/MS	
Toxicokinetic study	16 volunteers exposed to 80 mg/m^3 NMP for 8 h under either whole body, i.e. inhalational + dermal, or dermal only + influence of moderate physical workload on the uptake of NMP	NMP 5-HNMP 2-HMSI	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	CG-MS	

Study type	Study design	Metabolites	Results	Analytical method	Reference
Toxicokinetic study	6 male volunteers exposed for 8 h on 4 different days to 0, 10, 25 and 50 mg/m^3 NMP	Plasma NMP	The mean (range) concentrations of NMP in plasma at the end of exposure to 10, 25, and 50 mg/m^3 were 0.33 mg/l; 0.99 mg/l, and 1.6 mg/l; calculated urinary excretion factor 0.65		
NEP					
Toxicokinetic study	3 male volunteers, orally dosed 20.9 mg urine samples collected over 4 days post dose	5-HNEP 2-HESI	After 4 days 50.7 % of the dose of both metabolites in urine, 29.1 % of 5-HNEP and 21.6 % of 2-HESI were recovered. 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 now 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.	GC/ MS and the target metabolites were quantified by isotope dilution	

Table A 6: Biomonitoring levels for NMP and metabolites

Country and reference	Type of Work	Airborne exposure (mg/m³)		5-HNMP (mg/L)		2-HMSI (mg/L)		Remarks	Analytical method
		sample n	mean; (range)	sample n	mean; (range)	sample n	mean; (range)		
Germany; Ulrich et al., 2018	Biobank samples (ESB) - Pop$_{gen}$			540	34.7[GM, d]; (LOQ-386[d])	540	45.3[GM, d]; (LOQ-538[d])	5-HNMP and 2-HMSI identified in 98% and 99.6% of samples; years: 1991 to 2014; students 20-30 years;	stable isotope dilution analysis using solid phase extraction followed by derivatisation and CG-EL-MS/MS
Germany; Schmied-Tobies et al., 2021	GerES V-Pop$_{gen}$			2178	0.074; (0.022-0.18[c])	2213	0.053; (0.023-0.1[c])	Children and adolescents. age 3-17; from 2014-2017	GC-MS
Germany; Schindler et al., 2012	Unexposed Pop$_{gen}$			56	0.07[a]; (LOD-0.62)	56	0.064[a]; (LOD-0.25)		GC-MS

Country and reference	Type of Work	Airborne exposure (mg/m³)		5-HNMP (mg/L)		2-HMSI (mg/L)		Remarks	Analytical method
		sample n	mean; (range)	sample n	mean; (range)	sample n	mean; (range)		
Finland (Porras et al. 2009)	pilot study among paint manufacturers, graffiti removers and cleaners and lacquers	n=18	range <0.01-1.62 mg/m3 (8 h TWA)	n=10 (total), n=2-3 per work task	pre-shift/post-shift (µmol/l)* paint manufacturing 0.64±0,22/0.7±0,28 graffiti removal 21,7±23,9/ 23,3±10, cleaning 5,2±8,8/52,5 ± 17 parquet varnishing 16,9±13,5/42,1± 32,1	n=10 (total), n=2-3 per work task	pre-shift/post-shift (µmol/l) paint manufacturing 0 /0.26±0,27 graffiti removal 14,9±14,1/ 16,6±12,3, cleaning 1,08±0.88/4.48 ± 1.97 parquet varnishing 9,46±3,43/9,87± 5.96	Pilot study on workers; Sampling: pre-shift morning sample, post-shift, evening, next morning	LC-MS
Switzerland; Haufroid et al., 2014	Graffiti remover, Polymer, Cleaning Agents	91	0.18[a]; (0.002-6.99[b])	91	0.6[a]; (0.1-29.00[b])	91	0.8[a]; (0.2-23.3[b])		LG + tandem MS
Germany; Meier et al., 2013	Spraying department automotive industry			69	1.42; (0.12-13.43)	69	0.64; (0.09-2.87)		GC-MS

[a] median; [b] 5th-95th percentile; [c] 10th-95th percentile; [d] µg/g creatinine; [GM] Geometric mean; Pop_{gen} =general population;

NMP = N-Methyl-2-pyrrolidone; 5-HNMP = 5-hydroxy-N-methyl-2-pyrrolidone; 2-HMSI = 2-hydroxy-N-methylsuccinimide

***note that different unit is used**

Table A 7: Biomonitoring levels for NEP and metabolites

Country and reference	Type of Work	Airborne exposure (mg/m^3)		5-HNEP (mg/L)		2-HESI (mg/L)		Remarks	Analytical method
		sample n	mean; (range)	sample n	mean; (range)	sample n	mean; (range)		
Germany; Ulrich et al., 2018	Biobank samples (ESB) - Pop_{gen}			540	3.4[GM, d]; (LOQ-1061[d])	540	10.4[GM, d]; (LOQ-1019[d])	5-HNEP and 2-HESI identified in 34.8% and 75.7% of samples	stable isotope dilution analysis using solid phase extraction followed by derivatization and CG-EL-MS/MS
Germany; Schmied-Tobies et al., 2021	GerES V - Pop_{gen}			2199	0.023; (LOQ-0.14[c])	2179	0.03; (LOQ-0.15[c])	Children and adults from 2014-2017	GC-MS
Germany; Koslitz et al., 2014	Autobmobile varnisher			12	0.41; (0.03-4.31)	12	0.62; (0.03-4.04)		GC-MS
Germany; Schindler et al., 2012	Unexposed Pop_{gen}			56	<0.015[a]; (LOD-0.77)	56	<0.005[a]; (LOD-0.31)		GC-MS

[a] median; [b] 5th-95th percentile; [c] 10th-95th percentile; [d] µg/g creatinine; Pop_{gen} =general population

NEP = N-Ethyl-2-pyrrolidone; 5-HNEP = 5-hydroxy-N-ethyl-2-pyrrolidone; 2-HESI = 2-hydroxy-N-ethylsuccinimide

Table A 8: Biomonitoring levels for DMAC and metabolites acetamidomethyl)mercapturic acid

(Country) and reference	Type of Work	Airborne exposure (mg/m^3)		DMAc (mg/L)		NMA (mg/L)		Other metabolites			Remarks	Analytical methods
		n	mean; (range)	n	mean; (range)	n	mean; (range)	name (unit)	n	mean		
(Spies et al., 1995)	Acrylic fibre	419	2.01[b]	335	65.5[c]			U-MMAC U-ACET	335 335	24.5[c] 25.4[c]	A level of 35 mg MMAC/g creatinine in a postshift spot urine sample was recommended as a biomonitoring index.	
(Perbellini, et al., 2003)	Acrylic fibre			223	0.61; (0.05-3.15)		20.5[a,c] (1.5-173.6[c])				Unmodified DMAC and NMA concentrations in urine are good biomarkers for monitoring occupational exposure to the solvent	GC-MS
(Princivalle, et al., 2010)	Acrylic fibre						11.9[a,c]	AMMA		14.4[a,c]	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.	AMMA and NMA were determined by HPLC/MS and GC/MS

[a] median; [b] ppm 12-h TWA; [c] mg/g creatin DMAC = N,N-dimethylacetamide; NMA = , N-methylacetamide; MMAC = N-methylacetamide; ACET = acetamide; AMMA = S-(acetamidomethyl)mercapturic acid

Table A9: Biomonitoring levels for DMF

Country and reference	Type of Work	Airborne exposure (mg/m^3)		U-DMF (mg/L)		U-NMF (mg/L)		Other metabolites			Remarks	Analytical measures
		sample n	mean; (range)	sample n	mean; (range)	sample n	mean; (range)	Name (unit)	sample n	mean; (range)		
(Lareo and Perbellini, 1995)	Synthetic leather	54	16.4; (3-27)	50	0.45 (1)	54	23.3; (4-93)	U-AMCC (mg/l)	29	40.4; (2-117)		DMF, NMF: GC AMCC: GC/MS
(Käfferlein et al., 2005)	Polyacrylic fibre					35	10.2[a]; (1.6-59.7)	U-AMCC (mg/l)	35	11.3[a]; (0.6-116.5)		GC-TSD, GC-MS
								B-NMHb (nmol/g globin)	35	121.2[a]; (21.3-464.9)		
(Seitz et al., 2018)	Acrylic fibre	200	3.19[a]; (0.15-46.9)			201	4.8[a]; (0.2-50)	U-AMCC (mg/l)	181	6.73[a]; (0.05-89.2)		AMCC: SPE-LC-MS/MS NMF: GC-PND MCVal: GC-MS
								MCVal (nmol/g globin)	207	57.5[a]; (0.5-414)		
(Imbriani et al., 2002)	Synthetic leather		13.5[AM]; (0.4-75.2)			125	(0.5-114.2)	U-AMCC (mg/l)	125	(0.4-100.4)		AMCC: HPLC with UV detection

Country and reference	Type of Work	Airborne exposure (mg/m^3)		U-DMF (mg/L)		U-NMF (mg/L)		Other metabolites			Remarks	Analytical measures
		sample n	mean; (range)	sample n	mean; (range)	sample n	mean; (range)	Name (unit)	sample n	mean; (range)		
Korea; (Kim et al., 2004)	Synthetic leather	116	8.8[GM] ppm; (0.1-178.5 ppm)			143	47.5[GM]	U-AMCC (mg/l)	144	7.3[GM]	biological exposure limit for NMF (15 mg/ ml) was exceeded in 89.5% of urine samples, and 37.9% of air samples exceeded the environmental DMF exposure limit (10 ppm)	DMF: GC-FID NMF: GC-FTD NMF: GC-FTD
China; (Wang et al., 2014)	Sites near synthetic factories. Site:											GC-FID
	A	25	297.5			25[b]	7.7; (6.2)					
	B	39	430			39[b]	6.7; (2.7)					
	C	22	180			22[b]	1.5; (1.7)					
	D	23	565			23[b]	23.4;					

Country and reference	Type of Work	Airborne exposure (mg/m^3)		U-DMF (mg/L)		U-NMF (mg/L)		Other metabolites			Remarks	Analytical measures
		sample n	mean; (range)	sample n	mean; (range)	sample n	mean; (range)	Name (unit)	sample n	mean; (range)		
							(29.9)					
	E	24	270			24[b]	1.8; (0.8)					
Germany; (Käfferlein et al., 2000)	Polyacrylic fibre		1.76[a]; (0.1-159.77 ppm)			92	13.1; (0.5-108.7)	U-AMCC (mg/l)	92;	30.3; (0.5-204.9)		GC-TSD
Germany; Wrbitzky and Angerer, 1998)	Polyacrylic fibre	118	12.5; (ND-115.2)			125	4.7[ab]; (0.4-62.3[b])					
Japan; (Miyauchi et al., 2014)	Synthetic resin											
	Summer	128	5.2			128	4.1[GM]					
	Winter	142	3[GM]			142	1.4[GM]					
USA;	NHANES - Pop_{Gen}							MCAMA			Samples from 2005 - 2016, MCMA was	LC-MS

Country and reference	Type of Work	Airborne exposure (mg/m^3)		U-DMF (mg/L)		U-NMF (mg/L)		Other metabolites			Remarks	Analytical measures
		sample n	mean; (range)	sample n	mean; (range)	sample n	mean; (range)	Name (unit)	sample n	mean; (range)		
(Kenwood et al., 2021)	smokers								8272; 0.298-0.8[b]; 0.072-0.2[b]; (0.517[ab], smokers); (0.127[ab]; non-smokers)		detected in > 98% of samples	
	non-smokers											
(Kenwood et al., 2021)	NHANES - PopGen							MCAMA			Samples from 2005 - 2016, MCMA was detected in > 98% of samples	
	smokers								8272	0.517[ab]; (0.298-0.8[b])		
	Non-smokers									0.127[ab]; (0.072-0.2[b])		

Country and reference	Type of Work	Airborne exposure (mg/m^3)		U-DMF (mg/L)		U-NMF (mg/L)		Other metabolites			Remarks	Analytical measures
		sample n	mean; (range)	sample n	mean; (range)	sample n	mean; (range)	Name (unit)	sample n	mean; (range)		
USA; (Jain, 2015)	NHANES - PopGen							U-AMCC (mg/L)	402		Samples from 2011-2012 (aged 12-19)	
	males									0.96[GM]		
	females									1.08[GM]		

[a] **median;** [b] **mg/g creatinine DMF =** N,N-dimethylformamide; **AMCC =** N-Acetyl-S-(N-methylcarbamoyl) cystein; **NMHb =** N-methylcarbamoylated haemoglobin; **MCVal =** N-methylcarbamoyl adduct at haemoglobin; **MCAMA =** Methylcarbamoyl mercapturic acid; **$Popg_{en}$ =general population**

4 Bisphenols full RA report

Risk assessment for bisphenols A and S (update results from the aligned studies)

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Acknowledgments

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and all HBM4EU aligned study data owners for providing the data that were used in this assessment: Esteban (France), POLAES (Poland), CPHminipub/DYMS (Denmark), DIET_HBM (Iceland), FinHealth (Finland), (C)ELSPAC (Czech Republic), CIPH (Croatia), INSEF-ExQAP (Portugal), Swiss TPH (Switzerland), ESB (Germany), Oriscav (Luxembourg)

4.1 Summary

A risk assessment was previously performed in deliverable D5.8 for bisphenols, namely bisphenol A and S, for which sufficient data were available and for which HBM-Guidance Values (HBM-GVs) were derived in Task 5.2.

Overall, there was a clear difference between risk assessment performed for BPA and BPS exposure, with very low RCR regarding exposure to BPA contrary to those obtained for BPS. However, this was due to the fact that the recommended HBM-GVs for BPS are based on endocrine disrupting health effects occurring in animals at very low doses, contrary to the values calculated for BPA based on the temporary tolerable daily intake (t-TDI) from EFSA in 2015.

The results from the aligned studies were used to update our previous assessment.

The outcome of this update, taking into account P95 levels measured in the aligned studies for adults of the general population, remains unchanged with regards to what has been concluded in D5.8, and results are consistent. The difference observed between risk assessment for BPA and BPS exposure is still due to the different choices related to the derivation for the HBM-GV_{GenPop}.

Still, the work performed exemplify how biomonitoring data can be used in the risk assessment of bisphenols and also challenges and uncertainties related to its use in bisphenols case.

4.2 Summary of the previous risk assessment for bisphenols A and S

A risk assessment was previously performed in deliverable D5.8 for bisphenols, namely bisphenol A and S, for which sufficient data were available and for which HBM-Guidance Values (HBM-GVs) were derived in Task 5.2. Bisphenol F was also addressed in task 5.2, but no HBM-GV could be derived and thus, no risk assessment could be performed for this substance at this moment.

HBM data were gathered from the HBM4EU data repository made available by WP10 of HBM4EU and a literature search was additionally performed. These HBM data were then compared to HBM-GVs to calculate Risk Characterisation Ratios (RCRs).

$$RCR = \frac{95th\ Percentile\ value}{HBM-GV}$$

Overall, there was a clear difference between risk assessment performed for BPA and BPS exposure, with very low RCR regarding exposure to BPA contrary to those obtained for BPS. However, this was due to the fact that the recommended HBM-GVs for BPS are based on endocrine disrupting health effects occurring in animals at very low doses, contrary to the values calculated for BPA based on the temporary tolerable daily intake (t-TDI) from EFSA in 2015. This t-TDI is still under re-evaluation and if a new TDI based on low doses effects is proposed, the outcome of our risk assessment would change, and risk may not be ruled out, especially for sensitive population such as pregnant women and young children. Levels of confidence associated to the HBM-GV_{GenPop} and HBM-GV_{worker} for BPS were considered as ‘medium/low’ within Task 5.2 of HBM4EU, while for the BPA the HBM-GV_{GenPop} was associated with a ‘medium’ level of confidence. The conclusions of ongoing initiative regarding both BPA and BPS, as well as new data, could strengthen or change the outcome of the risk assessment proposed in the D5.8 document.

Regarding the occupational field, the assessment of available HBM data indicated that the risk from occupational exposure to BPA and BPS should not be disregarded. Nevertheless, there is a clear need for HBM data and studies to be carried out for bisphenols, across Europe, and for various professional activities and sectors.

4.3 Methodology

The risk assessment performed in D5.8 for bisphenols was updated with HBM data obtained from the HBM4EU aligned studies in the general population.

Total bisphenol A was measured in adults included in 10 aligned studies from 10 different European countries. Total bisphenol S was also measured in those aligned studies except for the Finnish FinHealth study.

The 95th percentiles (P95) reported in the aligned studies were compared to the corresponding HBM-GVs$_{GenPop}$ for BPA and for total BPS in urine to calculate the corresponding RCR. The HBM-GV$_{GenPop}$ for urinary total BPA was of 230 µg/L (Ougier et al. 2021), while for urinary total BPS the HBM-GV$_{GenPop}$ recommended is of 1.0 µg/L (Deliverable 5.9). No HBM-GV for BPF has been derived since the last risk assessment performed in D5.8. Therefore, it was still not possible to assess the risk for adults exposed to BPF included in the HBM4EU aligned studies.

4.4 Risk characterisation and uncertainty analysis

The outcome of the risk assessment taking into account P95 levels measured in the aligned studies remains unchanged with regards to what has been concluded in D5.8. Considering the HBM-GVs$_{GenPop}$ derived, the very low RCR calculated for total bisphenol A (Table 1) suggest that the risk can be ruled out for the sampled population in all the aligned studies, while results for BPS suggest that there is a concern for sampled population with all RCR being superior 1, except for the Polish and German studies (Table 2).

Table 10: Summary of HBM data from HBM4EU aligned studies for total BPA in the general population

Cohort	Country	N	Type of sampling	HBM-GV$_{GenPop}$ Total BPA in urine (µg/L)	P95 (µg/L)	RCR
Esteban	France	163	First morning urine	230	9.24	0,04
POLAES	Poland	228	Spot	230	9.94	0,04
CPHminipub/DYMS	Denmark	287	Spot	230	6.62	0,03
DIET_HBM	Iceland	198	Spot	230	6.28	0,03
FinHealth	Finland	300	Spot	230	5.65	0,02
(C)ELSPAC	Czech Republic	290	First morning urine	230	8.74	0,04
CIPH	Croatia	300	First morning urine	230	8.39	0,04
INSEF-ExQAP	Portugal	296	First morning urine	230	11.68	0.05
Swiss TPH	Switzerland	300	First morning urine	230	2.52	0,01
ESB	Germany	180	24h	230	2.66	0,01
Oriscav	Luxembourg	209	Spot	230	13.03	0,06

Table 11: Summary of HBM data from HBM4EU aligned studies for total BPS in the general population

Cohort	Country	N	Type of sampling	HBM-GV_{GenPop} Total BPS in urine (µg/L)	P95 (µg/L)	RCR
Esteban	France	163	First morning urine	1.0	6.93	6.9
POLAES	Poland	228	Spot	1.0	0.49	0.5
CPHminipub/DYMS	Denmark	287	Spot	1.0	2.23	2.2
DIET_HBM	Iceland	198	Spot	1.0	1.55	1.5
(C)ELSPAC	Czech Republic	290	First morning urine	1.0	1.63	1.6
CIPH	Croatia	300	First morning urine	1.0	2.81	2.8
INSEF-ExQAP	Portugal	296	First morning urine	1.0	6.62	6.6
Swiss TPH	Switzerland	300	First morning urine	1.0	2.14	2.1
ESB	Germany	180	24h	1.0	0.22	0.2
Oriscav	Luxembourg	209	Spot	1.0	2.40	2.4

4.5 Discussion and conclusions

The risk assessment performed in Deliverable D5.8 aimed at addressing the following policy questions (questions 3, 4 and 6 of the scoping document on bisphenols):

- Are bisphenols exposure levels of concern for health?
- Is occupational exposure of cashiers a health concern?
- Are health risks age and gender dependent?

We concluded that the risk could be ruled out for the general population exposed to BPA, but it was not the case for bisphenol S with some RCR calculated suggesting exposures of concern. Regarding occupational exposure, available HBM data indicated that the risk from occupational exposure should not be disregarded, and that protective measures need to be taken regarding BPS exposure. Moreover, we recommended that more studies should be carried out for various occupational activities for all bisphenols.

In the aligned studies only measurements for adults in the general population were available, thus only the first of the policy question mentioned above could be addressed.

From the results of these aligned studies, the difference remains between risk assessment for BPA and BPS exposure and is due to the different choices related to the derivation for the HBM-GV_{GenPop} as already discussed in the deliverable D5.8.

Therefore, the results presented in the document highlight the importance of the different initiatives currently underway for both the re-evaluation of the EFSA's t-TDI for BPA and for assessing the health effects and levels of toxicity for BPS. The outcome of this work could lead to a strengthening or the re-assessment of one or both of the HBM-GVs_{GenPop} proposed for BPA and BPS, and of the levels of confidence associated. This could therefore change the conclusions of our risk assessment.

The EFSA Panel on Food Contact and Materials, Enzymes and Processing Aids (CEP) recently release for consultation a re-evaluation of their t-TDI set in 2015. According to this proposal, the new TDI would be established at 0.04 ng/kg bw/d of total BPA. This value is 10^5 times lower than the current t-TDI, the RCR calculated, in this document are only 10^2 times lower than 1. In fact, when recalculating the HBM-GVs for total BPA in urine for the general population, on the basis of this new TDI proposal, and by the means of the PBPK model by Karrer (2018), Ineris has obtained HBM-GV divided by 10^5 (2.3 ng/L for adults and 1.4 ng/L for children). Therefore, if this new TDI is to be confirmed after the consultation period, comparing the P95 for total BPA measured in the aligned studies to a HBM-GV derived from the new TDI proposed, would most likely result in RCR exceeding 1 by far. Thus, the risk for consumers related to BPA exposure would be of concern with regard to this new value proposed, and protective measures would need to be taken.

Still, the work presented in this document as well as in Deliverable D5.8 exemplify how biomonitoring data can be used in the risk assessment of bisphenols and also highlights challenges and uncertainties related to its use in bisphenols case. Moreover, the efforts to perform aligned studies in Europe are of utmost importance and must be continued to monitor on the long term the changes of the exposure of the general population in Europe to bisphenols A and S, as well as to their analogues.

4.6 References

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5 Diisocyanates full RA report

Risk assessment and environmental burden of disease calculation for diisocyanates

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5.1 Summary

Introduction

Diisocyanates are a group of chemicals containing two isocyanate functional groups (R–N=C=O, NCO). The most commonly used diisocyanates are 4,4'-methylene diphenyl diisocyanate (MDI), toluene diisocyanate (TDI) and hexamethylene diisocyanate (HDI). Due to the NCO functional groups, all diisocyanates induce similar health effects. MDI and TDI are classified as suspected of causing cancer (Carc. 2) according to the European Union Classification, Labelling and Packaging (CLP) regulation. In addition, MDI, TDI and HDI are classified as dermal and respiratory sensitisers, and as eye, skin and respiratory irritants. The Risk Assessment Committee (RAC) has identified respiratory health effects (occupational asthma, isocyanate sensitisation and bronchial hyperresponsiveness (BHR)) as the critical endpoints related to diisocyanate exposure. A threshold for BHR or for the development of asthma, could not be observed. However, based on the exposure-response studies on hyperresponsiveness or diisocyanate asthma, an occupational exposure limit value (OEL) could be defined as an 8-hour time weighted average (TWA) exposure based on the NCO groups.

Methodology

The MDI and TDI external (air) intake levels were estimated from HBM diamine data by a developed PBPK method. For HDI exposure reconstruction was based on a published correlation equation (Maitre et al. 1996). Unpublished Finnish Human Biomonitoring data (HBM) from the Finnish Institute of Occupational Health (FIOH) was the main data source used in the risk assessment, but urinary levels were also compared with published biomonitoring studies. To combine the exposure reconstruction estimates with excess BHR risk estimations, the data points as provided by RAC were used to fit a spline curve. The number of exposed workers to diisocyanates in Finland were estimated from the FIOH FINJEM database (Finnish job-exposure matrix) which covers the major occupational exposures in Finland since 1945 (Kauppinen et al. 2014). Further RAC (2020) estimations for the exposed workers were compared with the total number of Finnish workers in each sector.

Results

In general, excess risk is highest for MDI, especially for the construction sector where we retrieved an excess BHR risk of 3.5%. Also, for HDI and TDI the construction sector poses the highest risk: 2.9% and 3.2% accounting for 165 and 180 excess BHR cases in the Finnish worker population respectively. For the other sectors (the motor and vehicle repair sector, manufacturing of PUR products and assembling of industrial products) excess risk estimates were between 1.1 – 3.0 %.

Discussion

There are several uncertainties in the risk assessment. One issue is the generalisability of the Finnish dataset: in some cases, this data seems to be relatively low in comparison to published data, e.g., for MDI exposure and the manufacturing of PUR products industry. The same counts for TDI exposure in the assembler industry and for HDI in the motor vehicle repair industry. However, results from a recently conducted HBM4EU diisocyanate occupational study indicate that in general exposures were low, often even below the LOD for MDI. Furthermore, for HDI a correlation formula is used based on air HDI monomer and urinary HDA. In workplaces where prepolymers of HDI are used in coating applications, this may result in underestimation of exposure to reactive NCO groups coming from HDI prepolymers, which are not reflected in elevated HDA levels. An advantage of use biomonitoring data over external exposure data is that the HBM includes potential dermal exposure and/or the use of respiratory equipment and as such

provides a more realistic exposure dose. For using diisocyanates biomonitoring data we should pay attention to the sensitivity of analysis methods to be able to detect even lower HBM levels of these compounds to efficiently monitor the exposure. The exposure levels of diisocyanates can be anticipated to be lower in the future since the new regulation/restriction in EU (EC 2020/1149) about working with diisocyanates has been put into force. However, there is still a need to monitor the occupational exposure to diisocyanates because a threshold limit value for BHR cannot be established.

5.2 Introduction

Diisocyanates are a group of chemicals containing two isocyanate functional groups (R–N=C=O, NCO) in otherwise varied structures. Due to the functional groups, all diisocyanates induce similar health effects, and are potent skin and respiratory tract sensitisers. Most commonly used diisocyanates are 4,4'-methylene diphenyl diisocyanate (MDI), toluene diisocyanate (TDI) and hexamethylene diisocyanate (HDI). MDI and TDI are classified as suspected of causing cancer (Carc. 2) according to European Union Classification, Labeling and Packeting (CLP) system. In addition, MDI, TDI and HDI are classified as dermal and respiratory sensitisers, and as eye, skin and respiratory irritants (Basketter et al. 2017; RAC 2020).

Within HBM4EU a number of policy-related questions were derived:

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?

The first two questions were studied by Scholten et al. (2020), who provided an overview of available diisocyanate biomonitoring studies, and information on available markers to identify exposure to diisocyanates. In this report we are investigating the fourth question: to study human health impacts at current occupational diisocyanate exposures.

Recently the Risk Assessment Committee (RAC) published an opinion on diisocyanates (RAC, 2020). RAC identifies respiratory health effects (occupational asthma, isocyanate sensitisation and bronchial hyperresponsiveness) as the critical endpoints related to diisocyanate exposure. A threshold for bronchial hyper-responsiveness (BHR) or for the development of asthma, could not be observed.

However, based on the exposure-response studies on hyperresponsiveness or diisocyanate asthma by Pronk et al. (2009) and Collins et al. (2017), an OEL could be defined as an 8-hour time weighted average (TWA) exposure based on the NCO groups (Table 1). More information on these studies is detailed below in chapter 3.

Table 1: Excess risk of hyperresponsiveness or diisocyanate asthma, over a working life period (table taken from RAC, 2020)

Excess risk over a working life period	Exposure - response relations derived from Pronk et al. (2009), and Collins et al. (2017), in µg/m³ NCO in air
0.1%	<0.025
0.5%	0.027-0.040
1%	0.055-0.070
2%	0.12-0.19
3%	0.22-0.33
4%	0.40-0.48
5%	>0.67

Further RAC concludes that both inhalation and dermal exposure are likely and relevant routes for occupational exposure to diisocyanates and that both routes are relevant for induction of respiratory sensitisation (Redlich and Herrick 2008; Engfeldt et al. 2013)[7]. But they also conclude that the contribution of dermal exposure to respiratory sensitisation cannot be quantified at present.

5.3 Methodology

5.3.1 General approach

Figure 1 illustrates the proposed methodology for the diisocyanate risk assessment. We use a Finnish biomonitoring dataset, convert the reported levels to external dose (µg NCO/m^3) by using a physiological based kinetic (PBK) model, and compare the estimated levels to the exposure-excess risk relation (EERR) of the Risk Assessment Committee (RAC). We then proceed with a health impact assessment (HIA) by using estimated numbers of workers exposed to estimated levels.

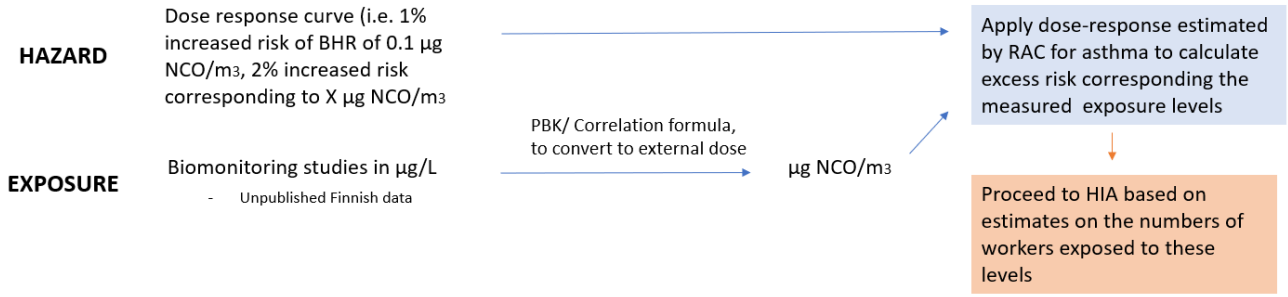

Figure 3: Methodology for diisocyanate Human Biomonitoring risk assessment

Our main dataset is the (non-published) Finnish dataset (as presented in chapter 4.2). We also use published biomonitoring studies (taken from Scholten et al. 2020) to compare to the Finnish dataset.

[7] Redlich CA, Herrick CA. Lung/skin connections in occupational lung disease. Curr Opin Allergy Clin Immunol 2008; 8: 115-9. Engfeldt M, Isaksson M, Zimerson E, Bruze M. Several cases of work-related allergic contact dermatitis caused by isocyanates at a company manufacturing heat exchangers. Contact Dermatitis 2013; 68: 175-80.

In general, biomonitoring studies often report urinary amines for specific diisocyanates, i.e. HDA, MDA and TDA, reflecting exposure to HDI, MDI and TDI respectively. Since the ERR is based on an NCO group approach (because diisocyanates share a common mechanism of inducing hypersensitivity reactions and there is not enough data to assess differences in potency for different diisocyanates), urinary amine concentrations are expressed as NCO group equivalent.

This is based on the following formula:

µg [NCO] / m^3 = µg [isocyanate] / m^3 x [molecular weight NCO-groups / [total molecular weight]

The molecular weight of the NCO groups is 84, and the total molecular weight is 174.2, 168.2, 250.3 for TDI, HDI and MDI respectively.

For conversion from µg/l to µmol/mol cr. we assumed a creatinine value of 12 mmol/l and a MW of TDA, HDA or MDA of 122.17, 179.22 gr/mol respectively. As an example, for TDA, 30 µg/l results in ~20 µmol/mol cr.

5.3.2 Exposure reconstruction

5.3.2.1 Biomarker distributions

We used the quantiles provided in Table 2 to estimate empirical urinary biomarker distribution functions for MDI, HDI and TDI, using linear extrapolation between quantiles. We subsequently drew 10,000 samples from these distributions for exposure reconstruction. Mean, median, 10^{th} and 90^{th} percentile of these samples are provided in Table 5.

5.3.2.2 PBK model for TDI and MDI

For reverse dosimetry TNO/IRAS developed a PBK model for TDI and MDI.

Parameters for the TDI and MDI PBPK model are described in detail in Table A1 (Annex) and a schematic overview of the model is included in Figure A1 (Annex). The model output is compared to published aggregated data and will be submitted for publication soon. Briefly about model kinetics: it is assumed that, after inhalation, about 20 % of TDI or MDI is absorbed, with the rest probably deposited in the lower and upper airways after which it may be transported back to the throat, swallowed, and excreted via feces (Timchalk et al. 1994). Diisocyanates are extremely reactive and react with smaller proteins or albumin (Kennedy et al. 1994). Diisocyanates conjugated to smaller proteins are readily excreted by the kidneys (first excretion phase), while diisocyanates conjugated to albumin may circulate in the body for weeks (with a half-life of 21 days) and are only excreted in urine after degradation into smaller molecules (Decos, 2019). This two-phase urinary elimination pattern is also described in practice, with the first phase being related to the more recent exposure and the second, much slower one probably related to release of TDA in urine from TDI adducts in the body (Lind 1996). There are several chemical specific parameters, and for most of these we have only rather imprecise estimates because of a lack of human kinetic data. These include amongst the most important: the proportion of TDI that is absorbed and the proportion of absorbed TDI that is bound to albumin versus that bound to smaller proteins and macromolecules that are excreted more readily. Both parameters directly affect the estimated amount of amines excreted in urine directly after exposure or the amount that accumulates in the body. Information on these parameters can be derived, at least theoretically, from volunteer studies. The uncertainty in the model parameters is accounted for by their parameter distribution: the more uncertain, the larger the appointed distribution.

Further we see in Human Biomonitoring studies that MDI is more slowly excreted than TDI. The biological basis is not yet clear, it could be due to different binding affinity to glutathione in the lungs, for example. For now, the major difference in the PBK model settings between MDI and TDI is the retention time in the lungs, which is slower for the first mentioned. This is substantiated by

the study of Gledhill et al. (2005) who reported that "*the reactivity of MDI is such that it may not be directly absorbed into systemic circulation from the lung, rather, it is highly likely that it reacts initially with the high glutathione content of the lung to form glutathione conjugates which act as a carrier"*.

Note that the PBK model is not applied to HDI because in general exposure to HDI involves mostly exposure to the oligomers (uretdione, biuret, and isocyanurate - up to 99%), and not the HDI monomers (Bello et al. 2020)[8]. Robbins et al. (2018)[9] indicate that "b*iological monitoring to estimate the systemic doses of HDI monomer and oligomers through exposure has been limited primarily to 1,6-diaminohexane (HDA), the hydrolysis product of HDI monomer, in urine and blood. However, it has been shown that* measured biomarker levels of HDI monomer exposure do not correlate with HDI oligomer *exposure"[10].*

The model is programmed such that it can account for uncertainty in parameter distributions. Exposure reconstruction is performed using a Bayesian Metropolis Hastings Monte Carlo (MHMC) algorithm, using an uninformative positive prior. We assumed an adult man of 75 kg, and that urine samples were collected post shift at steady state exposure levels, and exposure lasted all day. Further we used the available distribution information (i.e., the reported range) to provide an estimate of the distribution.

5.3.2.3 Formula for HDI and HDA

For deriving a HDI biological tolerance value (BAT value), the study by Maître et al. (1996) has been utilised (DFG 2017). A regression equation between HDI in the air and HDA in urine has been presented by Maître et al. (1996). The range of HDI concentrations in the study of Maître et al. (1996) was 0.3-97.7 µg/m^3 and the range of urinary HDA concentrations was 1.36-27.7 µg/g creatinine. The regression equation of Maître et al. (1996) is expressed by:

$\log_{10}$ (HDA) = 0.4396 × $\log_{10}$ (HDI) + 0.4612

And for reverse calculation of HDI concentration:

$\log_{10}$ (HDI) = ($\log_{10}$ (HDA) – 0.4612) / 0.4396

For example, the German MAK value for HDI is 35 µg/m^3. From this equation, 14 µg HDA/g creatinine can be calculated. On this basis, a BAT value of 15 µg HDA (after hydrolysis)/g creatinine was established. Sampling should be carried out at the end of exposure or end of shift (DFG 2017).

5.4 Hazard assessment

RAC (2020) used the exposure-response curve from Pronk et al. (2009) and Collins et al. (2017) for bronchial hyper-responsiveness and the development of asthma for deriving an exposure-excess risk relation (Table 1). Below these studies are described in more detail.

The studies by Pronk et al. (2007, 2009) investigated multiple health endpoints in a large group of 581 car body repair shop workers and industrial spray painters. Exposure to diisocyanates was studied by using LC-MS for isocyanate monomers, oligomers and products of thermal degradation. Short term exposure measurements on task level were converted to personal exposure estimates for each participant over a month, by using average time activity patterns. The researchers

[8] Bello A, Xue Y, Gore R, Woskie S, Bello D (2020). Exposures and urinary biomonitoring of aliphatic isocyanates in construction metal structure coating. Int J Hyg Environ Health. 226:113495.
[9] Robbins Z, Bodnar W, Zhang Z et al. (2018) Trisaminohexyl isocyanurate, a urinary biomarker of HDI isocyanurate exposure. J Chromatogr B Analyt Technol Biomed Life Sci; 1076: 117–29.
[10] There is one study on a biomarker for isocyanurate but there is no further data for this biomarker. This biomarker is also not investigated in the field study.

included BHR in their analysis (which DECOS considers most predictive for the development of occupational asthma). All associations were adjusted for sex, atopy, age and current smoking status. The study limitations include potential bias due to the healthy worker effect, and some workers (the industrial spray painters) used protective equipment.

Collins et al. (2017) collected surveillance data over several years from three plants in the United States. In total 197 workers TDI producing facilities were monitored from 2007 until 2012. Exposure for different groups was estimated based on TDI air concentrations and questionnaires. Based on the gathered data Collins et al. (2017) report an exposure response relation for TDI-induced asthma. A limitation of the study is possible misclassification since the cases of asthma were not formally clinically diagnosed by the consulting pulmonologist.

5.5 Exposure assessment using Human Biomonitoring data

5.5.1 Finnish data

Biomonitoring data from Finnish Institute of Occupational Health (FIOH) occupational diisocyanate screening studies from years 2008-2021 were gathered for specific sectors (Table 2). This Finnish data set will be published separately in a manuscript under preparation by Huuskonen et al. (manuscript).

The biomonitoring data originated from a register of biomonitoring measurements upheld by the FIOH, according to the law on the activities and funding of FIOH (STM 159/1978). This law defines the information gathered in the FIOH databases and gives FIOH permission to use the gathered data for research purposes. The database, which is not publicly available, consists of 754 samples from 2008–2021, sent to the Institute for exposure monitoring by occupational health care units. From these, specific sectors were identified, and data related to those extracted to calculate exposure in specific sectors using diisocyanates. Informed consent, including a consent to store the measurement results to the FIOH database, was obtained from all workers providing samples for analysis. Contextual information stored to the database includes sample timing, sex, smoking information, job titles and company information. It is unclear if the workers wore RPE.

Urine samples of workers were taken post-shift in the end of week or working period. Dermal exposure was not collected. The measured diamine samples below LOQ were treated as LOQ / 2 in the calculations.

Table 2: Biomonitoring data from Finnish occupational studies during 2008-2021 on diisocyanates for specific sectors.

Sector	Diisocyanate (n of urine samples)	Biomonitoring data (µmol/mol creatinine) expressed as range (GM; AM) and percentiles
Construction	MDI (53)	Urine MDA: 0.016 – 1.72 (0.16; 0.26) P50: 0.16, P75: 0.28, P95: 0.86
	TDI (6)	Urine TDA: 0.013 – 6.6 (0.14; 1.17) P50: 0.1, P75: 1.73, P95: 4.98
	HDI (7)	Urine HDA: 0.2 – 121.0 (1.2; 18.7) P50: 0.2, P75: 6.6, P95: 86.8
Motor and vehicle repair (MVR)	MDI (55)	Urine MDA: 0.01 – 9.9 (0.12; 0.33) P50: 0.1, P75: 0.12, P95: 0.63
	TDI (40)	Urine TDA: 0.01 – 5.6 (0.1; 0.25) P50: 0.1, P75: 0.1, P95: 0.85
	HDI (50)	Urine HDA: 0.02 – 49.0 (0.3; 2.6) P50: 0.2, P75: 0.2, P95: 27.8
Manufacturing PUR products/ polyurethane industry and rigid foam production	MDI (82)	Urine MDA: 0.02 – 2.3 (0.13; 0.19) P50: 0.1, P75: 0.12, P95: 0.6
	TDI (70)	Urine TDA: 0.013 – 13.3 (0.15; 0.86) P50: 0.1, P75: 0.1, P95: 9.0
	HDI (35)	Urine HDA: 0.03 – 13.3 (0.36; 1.5) P50: 0.2, P75: 0.23, P95: 11.7
Assemblers of industrial products	MDI (176)	Urine MDA: 0.02 – 12.4 (0.12; 0.25) P50: 0.1, P75: 0.19, P95: 0.58
	TDI (96)	Urine TDA: 0.01 – 75.5 (0.06; 1.0) P50: 0.05, P75: 0.1, P95: 1.3
	HDI (88)	Urine HDA: 0.02 – 76.0 (0.14; 1.6) P50: 0.1, P75: 0.2, P95: 5.3

AM: arithmetic mean; GM: geometrical mean; P: percentile

The Finnish diisocyanate HBM samples (Table 2) from construction sector consisted of urethane applicators, element workers, painters, and insulators of plumbing. The sector of motor and vehicle repair included mostly vehicle painters but also mechanics and vehicle-body repairers. The sector of manufacturing PU and plastic products contained PU and plastic founders, and laminators. The assemblers of industrial products had workers from shoe, door, tool and machine industries.

5.5.2 Available published studies

Scholten et al. (2020) published an overview of available biomonitoring studies from year 2000 onwards. This review was used to select studies that reported urinary amines (Table 3). Studies reporting new biomarkers, such as the study by Robbins et al. (2018) on urine TAHI, were not considered.

In general, most studies showed a fair correlation between urinary amines levels and airborne measurements. However, in some cases where dermal exposure was likely or RPE was used, the correlation was weaker (Scholten et al. 2020).

Table 3: Biomonitoring studies on diisocyanates for specific sectors *geometric mean (rather than median).

Sector	Study Populations (Country, no. workers)	Diisocyanate	Biomonitoring data expressed as range (median)	References
Construction	Switzerland*, 65	MDI	Urine: MDA 0.003- 3.2 µg/L [~0.001 – 1.3 µmol/mol cr.] The median is 1.340 nmol/l [0.11 µmol/ mol cr.]	Sabbioni et al. 2007
	Finland, 21	MDI	Urine: <0.1-0.2 µmol/mol cr. Dermal: 88% <2µg MDI/10cm2 on hand	Henriks-Eckerman et al. 2015
MVR	USA, 48	HDI	Urine HDA: <0.04-65.9 µg/L (GM 0.10, AM 0.54 µg/L) [~0.03 – 47.2 µmol/mol cr.]	Gaines et al. 2010
	UK, 995	HDI	Pre intervention: 1.34 (P90) µmol/mol cr. Intervention: 0.60 (P90) µmol/mol cr. Post intervention: 0.68 (P90) µmol/mol cr.	Jones et al. 2013
	Netherlands, 55 (10 workers from industrial paint shop)	HDI	Urine HDA: <2.9 – 146.5 µmol/mol cr. Median: 21.5	Pronk et al. 2006
	Finland* (n=6, car repair)	TDI	Sum-TDA(U) <0.02–0.76 (AM: 0.23) µmol/mol cr.	Rosenberg et al. 2002

Sector	Study Populations (Country, no. workers)	Diisocyanate	Biomonitoring data expressed as range (median)	References
Manufacturing PUR products/ polyurethane industry	UK, 71	HDI	Urine HDA: 56 < LoD, 13 > LoD. 9 > BMGV <0.5–10.1 (1.8) µmol/mol cr. Mean: 3.4 umol/mol creat (SD: 3.4), of those above the LOD!	Cocker et al. 2009
	France, 169	MDI	Urine: <0.1–23.6 µg/l [<0.5–19.25 µmol mol−1 cr.] AM: 1.25 ug/l [0.54 umol/mol creatinine]	Robert et al. 2007
	Sweden, 18	MDI	Urine: 0.3–78 (median: 2) µg/l [~0.13–32.7 (median: 0.8) µmol/ mol cr.]	Sennbro et al. 2006
	UK, 71	MDI	Urine: <0.5–0.7 µmol mol−1 cr no info on AM	Cocker et al. 2009
	UK, 90	MDI	Urine: 56/326 > LoD, 90% 0.5 µmol mol−1 cr. (median < LOD)	Keen et al. 2012
	Finland, 8	MDI	Nd – 0.13 umol/mol creatinine (AM: 0.07) (based on category: other PUR processes)	Rosenberg et al. 2002
Continuous / rigid foam production	Poland, 20	TDI	Sum-TDA (U) = <0.01–3.9 µmol mol−1 cr.	Swierczynska-Machura et al. 2015
	UK, 26	TDI	Sum-TDA (U) = <~0.4 to 7 (2.21) µmol mol−1 cr. (handlers)	Austin et al. 2007
	Belgium, 9	TDI	Sum-TDA (U) = 4.4–142.6 (18.01) µg l−1 [21 samples] [~3 to ~97 (~12.3) µmol mol−1 cr.]	Geens et al. 2012
	Finland, 17	TDI	Sum-TDA (U) = <0.05 to 39 µmol mol−1 cr.	Kaaria et al. 2001
	Sweden, n = 4 in 2000 and n=6 in 2005	TDI	2,4-TDA (U) ~ 0–10 µmol mol−1 cr. 2,6-TDA (U) ~ 0–35 µmol mol−1 cr.	Tinnerberg and Mattsson 2008
	Finland, 17	TDI	Sum-TDA(U) 0.2–39(4.9) µmol mol−1 cr	Säkkinen et al. 2011
	UK, 71	TDI	Sum-TDA(U) <0.5–15.5 (1.3) µmol mol−1 cr.	Cocker et al. 2009
	UK, 90	TDI	Sum-TDA(U) (µmol mol−1 cr.) <LOD)	Keen et al. 2012

Sector	Study Populations (Country, no. workers)	Diisocyanate	Biomonitoring data expressed as range (median)	References
	Finland, 57	MDI	Urine: 0.015–1.4 (0.13) µmol mol−1 cr.	Säkkinen et al. 2011
	Sweden, 18	MDI	Urine: 0.5–8.4 µg l−1 [~0.2–3.5 µmol mol−1 cr.]	Tinnerberg et al. 2014

5.6 Risk characterisation and uncertainty analysis

5.6.1 Number of exposed workers in specific sectors

The number of exposed workers to diisocyanates in Finland (Table 4) are estimated from the FIOH FINJEM database (Finnish job-exposure matrix) which covers the major occupational exposures that have occurred in Finland since 1945 (Kauppinen et al. 2014) and estimation of RAC (2020) against total number of Finnish workers in each sector. Some of the Finnish and RAC estimations of exposed workers in different sectors had slight differences. Therefore, a mean estimate of exposed workers to diisocyanates was calculated based on the generated range from FIOH and RAC data.

Table 4: Estimated number of exposed workers to diisocyanates in Finland

Sectors with exposure	Estimated number of workers	Estimated number of exposed workers (range, %)	Estimated number of exposed workers (mean, %)
Construction	25 300	2 500 – 9 000* (10-36)	5 700 (23)
Motor Vehicle Manufacture and Repair (Painting)	22 000	8 100* – 10 600# (37-48)	9 300 (42)
Assembly of Machinery and Electric Devices	14 600	1500* (10)	1 500 (10)
Furniture Manufacture	5 300	500* – 1 000# (10-19)	800 (15)
Plastic Industry	4 000	800# – 1 000* (20-25)	900 (23)
Total	71 200		18 200 (26)

* FINJEM/FIOH estimation, # RAC estimation

5.6.2 Exposure reconstruction results

The median and mean of the calculated air exposures is presented in Table 5. We did not include uncertainty of the PBK model (for MDI and TDI) because based on sensitivity analyses (not shown) this uncertainty was relatively unimportant and did not include major uncertainty components related to variability in exact exposure scenarios.

Table 5: Results from exposure reconstruction based on Finnish data.

Sectors	Diisocyanate	Mean, median, 10th and 90th percentile for the urinary biomarker samples (µmol/mol cr)	Exposure reconstruction (ug NCO/m^3) Mean, median, 10th and 90th percentile
Construction	MDI	0.28, 0.16 (0.05, 0.71)	0.61, 0.35 (0.12, 1.55)
	TDI	1.23, 0.10 (0.04, 4.23)	1.35, 0.11 (0.05, 4.64)
	HDI	15.75, 0.20 (0.20, 68.02)	257.12, 0.00 (0.00, 1012.62)
MVR	MDI	0.40, 0.10 (0.05, 0.50)	0.87, 0.22 (0.10, 1.08)
	TDI	0.32, 0.10 (0.03, 0.65)	0.35, 0.11 (0.04, 0.72)
	HDI	4.90, 0.20 (0.08, 21.25)	22.73, 0.00 (0.00, 71.78)
Manufacturing PUR products	MDI	0.21, 0.10 (0.06, 0.47)	0.46, 0.22 (0.12, 1.03)
	TDI	1.52, 0.10 (0.06, 6.79)	1.67, 0.11 (0.07, 7.46)
	HDI	1.99, 0.20 (0.16, 8.79)	2.26, 0.00 (0.00, 9.62)
Assemblers of industrial products	MDI	0.45, 0.10 (0.05, 0.48)	0.99, 0.22 (0.10, 1.05)
	TDI	2.19, 0.05 (0.01, 1.01)	2.40, 0.06 (0.01, 1.11)
	HDI	2.63, 0.10 (0.03, 4.01)	21.81, 0.00 (0.00, 1.61)

5.7 Environmental burden of disease (EBoD)

Excess risk of BHR is calculated based on the estimated distribution of air exposures (Table 6). The excess risk function was estimated by interpolating the exposure specific risk estimates provided in RAC (2020) using a spline function. Estimated excess risks beyond 7.5 % were capped at this level to avoid possible unrealistic extrapolation beyond the highest excess risk reported by RAC (5 %) (Table 1). The number of excess BHR cases for the sector of PUR manufacture was estimated by combining the exposed workers in plastic industry and furniture manufacture sectors.

Table 6: Estimated excess risk of developing bronchial hyperresponsiveness based on diisocyanate exposure in specific sectors in Finland.

Sectors	Diisocyanate	Excess risk (%)	Number of excess cases of BHR
Construction	MDI	3.5	200
	TDI	3.2	180
	HDI	2.9	165
MVR	MDI	3.0	279
	TDI	2.1	195
	HDI	1.7	158
Manufacturing PUR products	MDI	2.9	49
	TDI	2.6	44
	HDI	1.5	26
Assemblers of industrial products	MDI	3.0	45
	TDI	1.8	27
	HDI	1.1	17

5.8 Discussion and conclusions

We calculated excess risk for diisocyanate exposure in various sectors. In general, excess risk is highest for MDI, especially for the construction sector where we retrieved an excess BHR risk of 3.5%. This indicates that for Finnish construction sector the expected excess number of BHR cases is 200. For HDI the motor and vehicle repair sector pose excess risk of 1.7 % and indicates 158 excess BHR cases in Finnish workers.

Our approach encompasses several steps which all contribute to uncertainty.

Biomonitoring studies

The main source of urinary data is the Finnish dataset. One might wonder to which extent this dataset is a good reflection of the European diisocyanate exposure. For the construction industry (MDI) the Finnish urinary data is higher in comparison to published biomonitoring studies (Sabbioni 2007, Henriks-Eckerman 2015). However, the Finnish MDI data seems to be relatively low in comparison to published data for the manufacturing of PUR products industry: there are five published studies in this sector of which two (Robert et al. 2007, Sennbro et al. 2006) report higher levels. According to the Finnish dataset the GM, AM and median are 0.13, 0.19 and 0.1 µmol/mol creatinine. Robert et al. (2007) reported an AM of 0.54 µmol/mol creatinine, whereas Sennbro et al. (2006) reported a median of 0.8 µmol/mol creatinine. For TDI there are eight published studies in the assemblers of industrial projects industry of which at least four (Austin 2007, Geens 2012,

Sakkinen et al. 2011, Cocker et al. 2009) reported much higher average amine levels in comparison to the Finnish dataset (Table 2).

In the MVR sector, HDA levels in studies by Gaines et al. (2010) and Jones et al. (2013) were at lower levels (median 0.1 and 0.6-1.34 µmol/mol, respectively) as the Finnish data (mean 4.9 and median 0.2 µmol HDA/g creatinine). However, data from Pronk et al. (2006) was much higher with median level of 21.5 µg HDA/g creatinine. We used the Finnish dataset because we had quantitative information on the quantiles so we could estimate an exposure distribution. The published data is often very limited.

Exposure reconstruction

To convert the biomonitoring data, we used a PBK model for MDI and TDI. The PBK model output is compared to published aggregated data, but not yet calibrated. For HDI, a correlation formula by Maitre et al. (1996) was used. The range of HDI concentrations in the study from Maitre et al. (1996) were 0.3-97.7 µg/m^3 and urinary HDA concentrations 1.36-27.7 µg/g creatinine. Values much outside those concentration ranges of the equation will give uncertain results. In addition, the correlation has been established for air HDI monomer and urinary HDA. In workplaces where prepolymers of HDI are used in coating applications, this may result in underestimation of exposure to reactive NCO groups coming from HDI prepolymers, which are not reflected as elevated HDA levels.

HBM4EU field study

In 2021 and 2022 a diisocyanate field study was conducted in five countries. The main industry fields covered in the HBM4EU diisocyanate study were the use of MDI in construction and in the use of MDI based glues, and the use of HDI and MDI in the motor vehicle manufacturing and repair.

Results are currently being processed but in general diisocyanate air levels in these sectors were below the binding occupational levels proposed in EU. Especially when MDI is used, the levels were often below the detection limits. The commercially available methods to detect dermal contamination were not effective to detect skin contamination.

Excess risk and HIA

To proceed to a HIA we used numbers of estimated exposed workers for each sector. These numbers are based predominantly on Finnish estimates. However, estimates are in general difficult to extract for different countries. The exposure levels to diisocyanates in the Finnish data were low in general which indicates also low excess risk for BHR. However, the exposure levels had a lot of variation especially at higher percentiles (e.g., P90 and P95) which are driven by few very high exposures. It has been demonstrated that diisocyanates can cause BHR even after short period of exposure which could explain excess asthma cases.

Dermal exposure

We did not consider dermal exposure because quantitative information is lacking. If dermal exposure was significant (and reflected in higher amine levels in urine), this would result in an overestimation of the external exposure. To be able to include dermal exposure in our PBK model we need to be able to calibrate the data with studies reporting both quantitative dermal and inhalation exposure, and urinary values. Those data are missing. Note that the studies by Collins et al. (2017) and Pronk et al. (2009) did not consider dermal exposure.

Advantage of using HBM data

Advantages of performing risk assessment by using HBM data are related to RPE use and skin exposure. When using RPEs during possible diisocyanate exposure the only way to ensure RPE effectiveness is to take biomonitoring samples, since air concentration measurements are not feasible. This also includes skin exposure of diisocyanates which contributes to asthma risk and can be monitored by HBM. For example, many paints and other coatings contain HDI which are used especially in motor and vehicle repair sector and cannot be applied without RPEs and protective clothing.

Future prospects

In the HBM of diisocyanates we should pay attention to the sensitivity of analysis methods to be able to detect even lower HBM levels of these compounds to efficiently monitor the exposure. The exposure levels of diisocyanates can be anticipated to be lower in the future since the new regulation/restriction in EU (EC 2020/1149) about working with diisocyanates has been put into force. However, there is still a need to monitor the occupational exposure to diisocyanates because a threshold limit value for BHR cannot be established.

Results in the light of policy question

PQ1. What is the current occupational exposure to diisocyanates?

The recently conducted HBM4EU occupational study on diisocyanate exposure found, in general, that diisocyanate air levels were below the binding occupational levels proposed in EU (Report on occupational studies, Deliverable Report D8.13, WP8: Targeted fieldwork surveys and alignment at EU level). Unpublished Finnish HBM data, which was the main data source used in the risk assessment, also indicated low exposure to diisocyanates. In some occupational sectors, the earlier published data estimate higher exposures. There is still a need to monitor the occupational exposure to diisocyanates because a threshold limit value for BHR cannot be established.

PQ4. What are the health risks and human health impacts of the current occupational diisocyanate exposures?

In general, the excess BHR risk estimated from Finnish data was highest for MDI, especially in the construction sector where an excess BHR risk of 3.5 % was estimated. This indicates that for the Finnish construction sector, the expected excess number of BHR cases is 200. Also for HDI and TDI, the construction sector poses the highest risk: 2.9 % and 3.2 % accounting for 165 and 180 excess BHR cases in the Finnish worker population, respectively. For the other sectors (the motor and vehicle repair sector, manufacturing of PUR products and assembling of industrial products) excess risk estimates were between 1.1 – 3.0 %.

5.9 References

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5.10 Annex

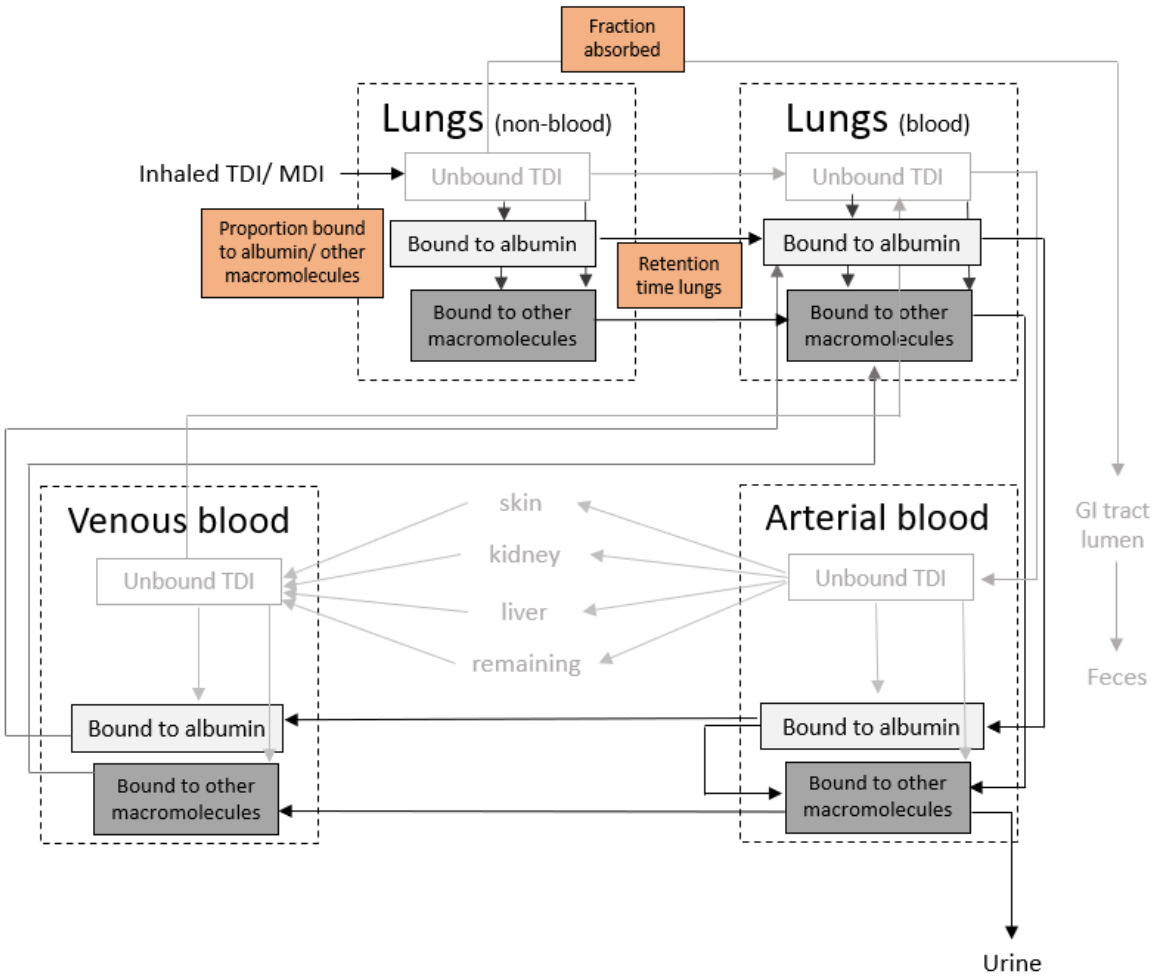

Figure A1: Schematic picture of physiological based kinetic model for diisocyanates (MDI and TDI).

Table A1: Parameter values for Diisocyanate physiologically based kinetic model (MDI and TDI)
*** Only MDI has a Vmax value due to hydrolysis to MDA and acetylation in liver compartment.**

Parameter	Abbreviation	Value	Distribution	Comments
Molecular weight	MW TDI	174.16	-	
	MW TDA	122.17	-	
	MW MDI	250.25	-	
	MW MDA	198.26		
LogP	LogP TDI	3.74	-	
	LogP MDI	0.00049	-	
Vapor pressure (Pa)	Pa TDI	1.3	-	
	Pa MDI		-	
Body weight (kg)	BW	75	Trunc Normal (60 – 90)	
Body length (cm)	BL	180	Trunc Normal (162 – 198)	
Breathing rate (l/hr)	Qbr	690	Trunc Normal (621 – 759)	
Cardiac output	Qc	390	Trunc Normal (351 – 430)	
Creatinine concentration in urine	Ccreat_ur	1 (g/L)	Trunc Normal (0.8 – 2.0)	[ICRP 2002]
Half-life of albumin (hours)	T_elim_alb	456	Trunc Normal (365 - 547)	
Urine production rate (cm3/kg/hr)	UPD	1.25	Trunc Normal (1.125 – 1.375)	Based on a BW of 75 kg [Davies 1993]
Glomerular filtration factor		0.7		Schwartz 1987. Pediatr Clin North Am. 34(3):571-90. doi: 10.1016/s0031-3955(16)36251-4.
Fraction absorbed (%)	Fr_abs	20	Uniform (0.1-0.3)	Based on voluntary studies by Brorson and Skarping (TDI), and the animal study by Gledhill (MDI)

Parameter	Abbreviation	Value	Distribution	Comments
Proportion bound to albumin (%)	PRC_albumin_macro_lung/ PRC_albumin_macro	0.2	Uniform (0.04 – 0.36)	Based on voluntary study by Brorson.
Metabolic conversion rate	Vmax*	10*		Based on 10% MDA in liver (Sepai 1995)
Concentration 50% conversion	Km	0.1E-09		Based on 10% MDA in liver (Sepai 1995)
Half life transfer from non-blood to blood in lungs (hours)	TDI - T_albumin_lun_bld/ T_macro_lun_bld/ T_lun_bld MDI - T_albumin_lun_bld/ T_macro_lun_bld/ T_lun_bld	0.08 14	Uniform (0.02 – 0.15) Uniform (2.8 – 25.2)	Based on the voluntary study by Budnik (2011)
Half-life of binding time to albumin/other macro molecules (hours)	MDI - T_on_albumin_macro_lung T_on_albumin_macro TDI - T_on_albumin_macro_lung T_on_albumin_macro	1.5 0.17	Uniform (0.3 – 2.7) Uniform (0.03 – 0.30)	Based on the voluntary study by Budnik (2011)

6 Lead full RA report

Environmental burden of disease calculation for lead (Pb):

A Slovenian case study

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Acknowledgments

In this assessment the aggregated data of the HBM4EU repository were used. We would like to akcnowledge the following data-owners and the following HBM studies: BIOAMBIENTES: Argelia Castaño, CzechHBM: Vladimira Puklova, GerESIV: Nina Vogel, FLEHSIII: Vera Nelen, FLEHSIV

6.1 Summary

The aim of this work was to estimate the environmental burden of disease (EBoD), expressed as disability-adjusted life years (DALYs) due to exposure to lead (Pb) in Slovenia (SLO) and selected EU countries. Data on blood lead levels (BLL) for children and adults at different ages or age groups were obtained from national Human Biomonitoring (HBM) surveys or similar studies. In Slovenia, these include the DEMOCOPHES and CROME research projects running from 2011−2012 and 2016, respectively; the first part of the national HBM-II survey in 2018 in Mura region, and an annual local HBM in the Meža valley (the Carinthia region), which is well-known Slovenian Pb pollution hot–spot (few centuries long Pb mining and processing). In adults, BLLs were obtained in a national wide HBM campaign (HBM-I, 2008−2014), including lactating *primiparous* mothers and men of the same age (18–49 years). In selected EU countries, BLLs were obtained from the HBM4EU repository (available through IPCHEM), including children and adolescents from campaigns in the Czech Republic (CzechHBM−CE_2016), Germany (GerES V in 2014−2017) and Belgium (Flanders; FLEHS IV in 2016−2020), while in adults, these include data from Spain (BIOAMBIENT.ES in 2009−2010), the Czech Republic (CzechHBM−AE_2015) and Belgium (Flanders; FLEHS I in 2004−2005). In children, DALYs were estimated for developmental neurotoxicity (lost cognitive development), based on a log-linear relationship between BLL and total numbers of Full–Scale IQ points (FSIQ) loss attributable to BLL above 20 µg/L. Considering no threshold in Pb exposure, the sensitivity analysis (using all ranges of BLL) was also performed. In adults, DALYs were estimated based on a more recent dose–response relationship and corresponding hazard ratios (relative risk) between BLL above 10 µg/L and premature mortality (all causes of deaths, ICD-10 code I to XX; above 20 years). The DALYs were presented in absolute numbers and per 100000. In the SLO case, DALYs were presented also as a mean and 95% confidence interval.

The highest proportion of children, having BLL above 20 µg/L, was identified in the Upper Meža valley hot−spot, e.g. 89% of children, aged 2−4 years, with BLL GM = 47 µg/L, followed by 88% children, aged 8−10 years from the same area, with BLL GM = 41 µg/L. For comparison, in the distant part of the valley, the Lower Meža valley, the proportion of children having BLL > 20 µg/L was 53%, with BLL GM = 34 µg/L. In addition, 10% of children in the Upper Meža valley had BLL above 100 µg/L and some of them also above 200 µg/L. In other SLO HBM studies, a small % (2−14%) had BLL above 20 µg/L with BLL GM between 22−33 µg/L, and none of children had BLL above 100 µg/L. In a recent Belgium (FLEHS IV) HBM study 0% of adolescents of 14−15 years had BLL above 20 µg/L. In the Czech Republic (CzechHBM−CE_2016), 5−17% of children had BLL above 20 µg/L, with BLL GM at 23 and 26 µg/L in age groups 6−11 and 3−5 years, respectively. In Germany (GerES V, 2014−2017), the proportions of children with BLL above 20 µg/L were as follows: 7% in the age group 3−5 years, 6% in the age group 6−10 years, 3% in the age group 11−13 years, and 3% in the age group 14−17 years. The corresponding BLL GMs were 25, 23, 22 and 25 µg/L, respectively. Corresponding DALYs in children were the highest in the Meža valley in general, 978−3437 (95%CI: 605−4955) per 100,000 children, which was more than 10−fold higher than in other Slovenian regions in general, 18−26 (95% CI: 11−37). This was also significantly higher than in children in selected EU countries. In the Czech Republic, DALYs were 19 and 127 (considering two age groups), and between 8 and 47 in Germany (considering four age groups). In general, DALYs were higher in younger children of 3−5 years, comparing children of age between 6 and 10 years and adolescents, 11-17 years. It is also obvious from the numerous studies that over time BLLs decreased significantly (e.g. FLEHS IV and GerES V).

In SLO adults, 18−49 years, 98% of participants in the Meža valley had BLL above 10 µg/L, with BLL GM at 28 µg/L. In Slovenia overall, this proportion was 92%, with BLL GM at 19 µg/L, and it was higher in men than in women, 21 and 18 µg/L, respectively. Corresponding DALYs were 464

(95%CI: 244−661) per 100,000 adults in the Meža valley versus 311 (95% CI: 161−450) in Slovenia overall. Regarding gender, DALYs were 348 (95%CI: 181−502) and 276 (95%CI: 142−399) in men and women, respectively. In Belgium (FLEHS I), 100% of participants of 40−59 years had BLL above 10 µg/L with BLL GM at 37 µg/L and corresponding DALYs was calculated at 733. In two age groups of adults in Spanish HBM, 97% of participants of 20−39 years had BLL above 10 µg/L with BLL GM at 21 µg/L, and 99% of participants of 40−59 years had BLL above 10 µg/L with BLL GM at 28 µg/L. Corresponding DALYs were 314 and 427, respectively. In two age groups of Czech's adults, 75% of participants of 20−39 years had BLL above 10 µg/L with BLL GM at 19 µg/L, while in the elder group of 40−59 years, 86% had BLL above 10 µg/L with BLL GM at 23 µg/L. The corresponding DALYs were 385 and 546, respectively. In general, DALYs were calculated higher in elder adults, and based on an older date HBM surveys (Belgium).

These estimates, however, should only be compared with caution, because of different time periods, differences in age groups of participants, and other potential differences, that could not be considered. These includes also some uncertainties related to dose response relationship used in the calculations and more studies are also needed to confirm this. Nevertheless, the results in this report can be considered as important, achieving one of the common goals of HBM4EU, e.g., harmonisation of the HBM protocols and consequently better understanding and comparison of human exposure and health outcomes at the EU level. New data on HBM at EU level, however, will certainly contribute significantly to a more reliable assessment of the EBoD.

6.2 Introduction

Lead (Pb) is an environmental contaminant and it has been classified by the IARC (in general) as possibly carcinogenic to humans (Group 2B) (IARC, 1987). Inorganic lead compounds have been classified as probably carcinogenic to humans (Group 2A) (IARC, 2006) and by the German Research Foundation (MAK Commission) in category 2 (to be regarded as human carcinogen). Organic lead compounds were not classifiable as to their carcinogenicity to humans (Group 3) (IARC, 2006). According to the ECHA Substance Infocard, Pb may damage fertility or the unborn child, causes damage to organs through prolonged or repeated exposure, is very toxic to aquatic life with long lasting effects, may cause cancer, is very toxic to aquatic life and may cause harm to breast-fed children. Pb is a substance of very high concern (SVHC) and included in the candidate list for authorisation. In addition, some uses of Pb are restricted under Annex XVII of REACH (https://echa.europa.eu/substance-information/-/substanceinfo/100.028.273).

Epidemiological studies have provided a lot of evidence for health effects at low levels of Pb in blood (< 5 µg/dL) and no thershold level was identified for any of them (EFSA, 2010; CDC, 2021). The German HBM Commission concluded that any setting of an "effect threshold" for blood lead levels (further as BLL) would be arbitrary and therefore unjustified. Based on the results of GerES III and IV, in combination with current data from the German Environmental Specimen Bank, the statistically derived reference levels were identified at 4 µg/dL for adult men, 3 µg/dL for adult women and 3.5 µg/dL for children (Lead Scoping Document–2019; https://www.hbm4eu.eu/wp-content/uploads/2019/03/HBM4EU_Scoping-Document_Lead_v1.0.pdf. 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 (EFSA, 2010).

Much of the lead toxicity facts and impacts to the human health have been already reviewed and described in the Lead Scoping Document–2019, mentioned above. It can be read in this document, that the results of BLLs surveys during the past two decades among the general population were

available in some European countries (see Scoping document, Table 1.1). Most of these studies also covered children's populations and decreasing trends in blood lead level of children were observed with lowering lead content of petrol and finally phasing out leaded petrol in various countries. During the past 5 years, BLL were available only for 7 countries (Belgium, Germany, Denmark, Kosovo, Poland, Slovenia and Sweden) as stated in the Scoping Document–2019.

There are few researches on Pb in Slovenian children. Some were performed in the framework of the international research projects, such as DEMOCOPHES (2011-12), including children between 6 and 12 years of age, and CROME (2016), including children 7−8 years of age (unpublished data). At the national level, the first such survey started in 2018 in the Mura region (Figure 1), including two age groups, 7−10 years, and 12−15 years (IJS, 2019). This survey continued in 2019 also in other regions, but it was interrupted in 2020 due to the coronavirus pandemic. Apart from these, at the local (regional) level in the Meža valley (the Carinthia region, Figure 1), regular blood biomonitoring has been performed in children between 1–9 years since 2004 (Ivartnik et al., 2015).

In adults, the first attempt to assess biological burden of Pb in Slovenia was probably the study of Eržen and Zaletel Kragelj in 2004 (Eržen and Zaletel Kragelj, 2004). They evaluated cadmium and lead burden from all sources (air, food, water) with the aim of obtaining initial information on cadmium and lead levels in blood of healthy and occupationally unexposed young males. In 2008−2010 in the Central Slovenia and Southeast region, the pilot national HBM-I survey started in adults, included lactating *primiparous* mothers and their male peers aged 18–49 years. In the period 2011−2014 the pilot phase was extended to the national wide HBM-I in all twelve statistical regions of Slovenia (Figure 1). The main aim was to obtain exposure estimates for selected environmental pollutants in this vulnerable population group, and at the same time allow estimation of newborn’s exposure through human milk, and the national reference values and to evaluate overall burden by different environmental chemicals (IJS, 2015; Snoj Tratnik et al., 2019). In all these studies, in children and in adults, the Meža valley was designated as a hot–spot. Particularly in that valley the risk of exposure to Pb and its adverse health effects is significantly increased because of mining and processing of Pb in the past few centuries, i.e., mine and lead smelter in the village of Žerjav (municipality of Črna na Koroškem). Pb emissions have been spreading from the industrial zone for many years and the soil in the valley is heavily loaded with lead. About 7000 inhabitants are therefore more exposed to Pb, which is particularly true for children and pregnant women as the most vulnerable group of the population. In the Meža valley Pb concentration in soil samples were often measured and found at 535 mg/kg (garden soil), 661 mg/kg (natural soil) and 1073 mg/kg (macadam), exceeding the set limit immission values for soil (Ivartnik et al., 2015). Other potentially polluted areas in Slovenia are more or less spread across the country and are due to past and current industrial activities, including the Upper Carniola region with an ironworks industry; the Central Sava river region with its coal–mining activities, thermo–power plant, cement plant, chemical company and glassware, and the Savinja river region, with the thermal power plant in city of Šoštanj, cement factory, metallurgy, chemical–processing company, ironworks and other large–scale industry (e.g. zinc factory in Celje). However, the potential food and drinking water exposure to Pb was not found to be higher than the EU average (Kirinčič et al., 2019).

The aim of this work was to calculate environmental burden of disease (EBoD) for Slovenian (SLO) population of children and adults, based on the HBM data for Pb from the past 5–10 years. The aim was also to compare the results of EBoD with the results in other countries, based on calculations using data from the HBM4EU repository (available through IPCHEM). The following endpoints for the EBoD calculations have been selected for these estimates:

- developmental neurotoxicity in young children (lost cognitive development) in children; and
- premature mortality (all–cause mortality) in adults.

The EBoD was expressed as disability-adjusted life years (DALYs), where one DALY represents the loss of the equivalent of one year of full health. DALYs for a disease or health condition are the sum of the years of life lost to due to premature mortality (YLLs) and the years lived with a disability (YLDs) due to prevalent cases of the disease or health condition in a population (http://www.who.int/entity/healthinfo/statistics/GlobalDALYmethods_2000_2011.pdf?ua=1).

Policy questions to be covered

To estimate the EBoD, the following policy questions were adopted from the Scoping document:

1. What is the concentration of lead in the human blood nowadays (after phasing out leaded petrol) in the countries of Europe?
2. What is the EBoD due to Pb exposure in children and adults, based on available HBM data (and potential comparison with other – selected– countries).
3. Do blood lead levels of both adults (especially pregnant women) and children still indicate permanent existence of lead exposure and what kind of exposure sources are the most important?

6.3 Methodology

6.3.1 Study populations and study areas

6.3.1.1 Children

For purpose of this work BLL data in SLO children, as obtained in different HBM surveys, was used and divided/grouped in four study groups as follows:

- **Study 1 (**DEMOCOPHES, 2011–2012); involved 95 children, aged 6–12 years from the Savinja region (56 children) and 39 children from Central Slovenia region;
- **Study 2** (CROME, 2016); involved 135 children, aged 7–8 years, with 122 children (90.4%) from Central Slovenia and 13 children (9.6%) from some other regions;
- **Study 3** (HBM-II in the Mura region, 2018); involved 135 children, aged 7–10 years (group 3a) and 94 teenagers, aged 12–15 years (group 3b). Results for these age groups were shown separately.
- **Study 4**. (local HBM survey in the Meža valley – hot spot, 2018–2020); involved 77 children, aged 8–10 years from the Upper Meža valley, UMV (group 4a), 195 children aged 2–4 years from the UMV (group 4b) and 200 children from the Lower Meža valley, LMV (group 4c). Results for these age groups were shown separately.

The results of the EBoD calculations for those studies (and groups) are presented in Table 1 and Table 2. Slovenian (statistical) regions are presented in Figure 1. In general, they were named mainly according to the rivers in these regions. The (river) Meža valley was noted as a hot spot in the Carinthia region.

6.3.1.2 Adults

For purpose of this work BLL data in Slovenian adult population was used from the national HBM survey. The study population in total (n=1084) included lactating *primiparous* mothers (n=535) and male participants (n=549), aged 18–49 years (average 29.9 years) from twelve statistical regions, with the Meža valley hot spot representing the region of Carinthia (Figure 1). For practical reasons, these twelve regions were combined into 10 study groups as follows:

- Group 1: Central+Littoral–Inner Carniola, n = 114;
- Group 2: Southeast Slovenia + Lower Sava, n = 158;
- Group 3: The Mura region, n = 88

- Group 4: The Drava region, n = 100
- Group 5: The Savinja, n = 160;
- Group 6: The Central Sava, n = 104;
- Group 7: Gorizia, n = 98;
- Group 8: Coastal–Karst, n = 100;
- Group 9: Upper Carniola, n = 83;
- Group 10: Carinthia – The Meža valley (Pb hot–spot), n = 79.

The results of EBoD calculations are presented (a) per group; (b) for all 10 groups combined (SLO total) and both genders; (c) for all groups combined (SLO total) excluding the Meža valley and both genders. The results are shown in Tables 3 and 4.

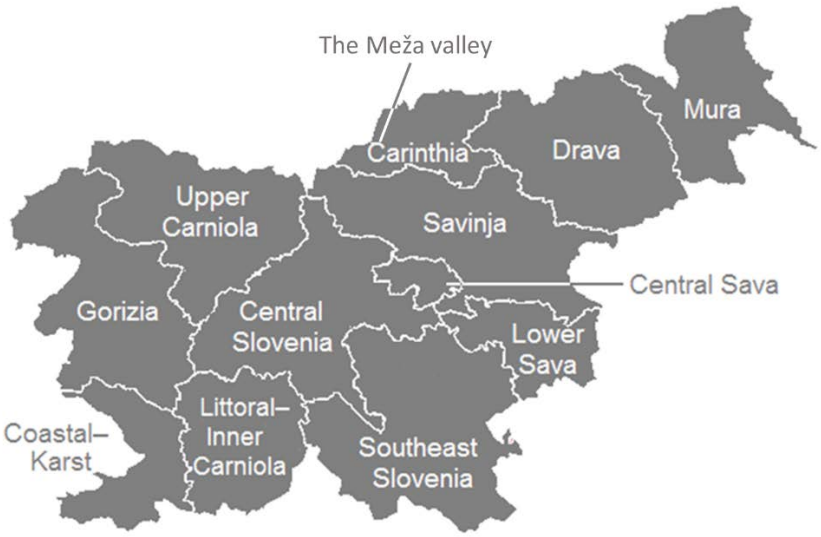

Figure 1: Statistical regions of Slovenia (Source: https://en.wikipedia.org/wiki/Statistical_regions_of_Slovenia). In the Carinthia region, the river Meža valley is designated as a hot–spot.

6.3.2 HBM data in selected EU countries

BLL data from the following HBM surveys was used from the HBM4EU repository (available through IPCHEM):

- Spain, BIOAMBIENT.ES, adults 20−39y, 2009−2010, n = 1121
- Spain, BIOAMBIENT.ES, adults 40−59y, 2009−2010, n = 709
- Czech Republic, CzechHBM-CE_2016, children 3−5y, 2016−2017, n = 159
- Czech Republic, CzechHBM-CE_2016, children 6−11y, 2016−2017, n = 252
- Czech Republic, CzechHBM−AE_2015, adults 20−39y, 2015, n = 140
- Czech Republic, CzechHBM−AE_2015, adults 40−59y, 2015, n = 148
- Germany, GerES V, children 3−5y, 2014−2017, n = 138
- Germany, GerES V, children 6−10y, 2014−2017, n = 231
- Germany, GerES V, children 11−13y, 2014−2017, n = 143
- Germany, GerES V, children 14−17y, 2014−2017, n = 208
- Belgium, FLEHS I adults, 40−59y, 2004−2005, n = 980
- Belgium, FLEHS IV adolescents, 14−15y, 2016−2020, n = 419

Based on a log-normal fit (Excel add-in: Crystal Ball) of aggregated data (50^{th} and 90^{th} percentiles; P50 and P90), the percentage of the subjects with more than 20 and 10 µg/L lead in blood was calculated in children and adults, respectively. Also, based on the distribution, the BLL (geometric mean, GM) to which these persons with BLL > 10 or 20 µg/L were exposed, were estimated, as shown in Tables 2 and 4 (see section 5).

6.3.3 Calculation basics

6.3.3.1 Children

For IQ loss in children the methodology of WHO was followed (Fewtrell et al., 2003). The total numbers of Full–Scale IQ points (FSIQ) loss in children (absolute and per 100,000 individuals) attributable to BLL above 20 µg/L was estimated based on widely accepted dose response functions between children's BLL and IQ (-1.88 IQ points for a duplication in BLL from 20 µg/L onwards; 95% Confidence Interval (CI): -1.16 to -2.59) (Lanphear et al., 2005). Based on the linear–log relationship proposed by Lanphear, a uniform decrease (i.e. linear relationship) was assumed over three ranges, for BLL between 20 and 100 µg/L; between 100 and 200 µg/L; and between 200 and 300 µg/L. The following estimated decrease in IQ points for an increase in BLL was used (Gould, 2009):

- -0.054 (95% CI: -0.034 to -0.075), for BLL between 20 and 100 µg/L;
- -0.019 (95% CI: -0.012 to -0.026), for BLL between 100 and 200 µg/L;
- -0.011 (95% CI: -0.007 to -0.015), for BLL between 200 and 300 µg/L.

There is a slight difference in the linear–log and the linear–interval dose response relationship at higher exposure levels, however, the linear–interval dose response relationship is more conservative in the lower dose range, i.e., BLL below 100 µg/L, as shown in Figure 2 (Remy et al., 2019).

Dose response estimates are lacking in the lower dose region, however a slope of -0.054 points/µg/L (95% CI: -0.034 to -0.075) could be applied to BLL between 0 and 20 µg/L as a sensitivity analysis (Bellinger et al., 2012). BLL at 20 µg/L was considered as a relevant action level (Gilbert and Weiss, 2006). This level was often used as a threshold, though the recent risk assessments have found that it was not possible to identify BLL below which no adverse effects

could be discerned (EFSA, 2010; CDC, 2021). The impact of Pb on IQ in the EBoD studies following described methodology was estimated in several EU countries (Remy et al., 2019; Roas–Rueda et al., 2019; Hänninen et al., 2014; Bierkens et al., 2012).

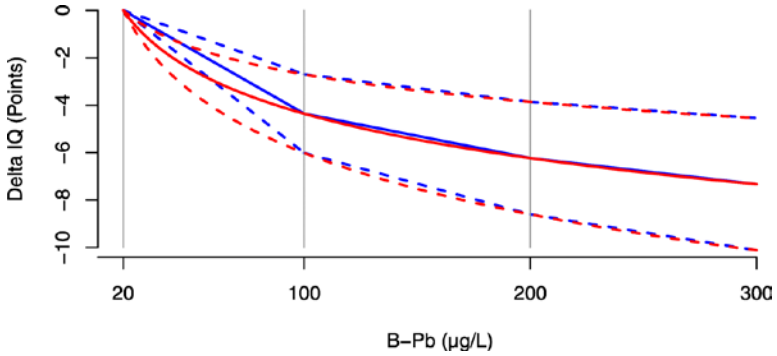

Figure 2: Dose response function: Inverse relationship between IQ and blood lead levels (BLL) for BLL between 20 µg/L and 300 µg/L. The red line represents the linear–log dose response relationship as estimated based on pooled international data by Lanphear et al. (2005). The blue line represents the linear–interval dose response relationship as suggested by Gould et al. (2009), which assumes uniform decreases between 20 and 100 µg/L, between 100 and 200 µg/L and above 200 µg/L. The dotted lines represent the 95% confidence limits for both dose response relationships. Source: Remy et al. (2019).

The methodology described below assumes children with exposure levels between 20 and 100 µg/L. In this case the UR -0.054 points/µg/L is valid. For other exposure ranges (above 100 µg/dL) the approach is similar but with according UR.

Average IQ loss

$$IQ_{loss} = E \times UR$$

where

'E' = exposure level above 20 µg/L (X - 20) of children exposed above 20 µg/L

'UR' = unit risk, -0.054(CI: -0.034 to -0.075) points/µg/L

Percentage of shift in IQ, IQ_{shift} to calculate the mild mental retardation (MMR)

IQ_{shift} (how many children will shift to an IQ below 70 due to exposure to Pb) was calculated using NORMDIST function (excel) considering the normal distribution of IQ points with a mean value of 100 IQ points and a standard deviation of 15 IQ points (Hänninen et al., 2014). Below an IQ of 70 mild mental retardation (MMR) is expected:

IQ_{shift} = NORMDIST(70+IQ_{loss};100;15;1) - NORMDIST (70;100;15;1)

The attributable number of cases per year, AC

$$AC_{-per\ year} = IQ_{shift} \times N \times P / 4$$

Where

'N' = number of children (0–4 year)[11] in population in the study area

'P' = proportion of children with BLL above 20 µg/L (%)

'4' = the assumption, that cases have occurred in the first year only. The burden of children having an age between 1–4 years was attributed to the first year (IQ loss calculated for children 0–4 years). BLL reported in Slovenian children (different ages, see section 2.1.1) were used as a proxy for BLL in children 0–4 years as was also done in other studies (Hänninen et al., 2014; Remy et al., 2019).

DALY / year

$$DALY_{per\ year} = AC \times f \times L$$

where

'f' = severity factor (disability weight) 0.36

'L' = duration of the condition (e.g. 75 or 80 years) for MMR as the health endpoint (Hänninen et al., 2014).

6.3.3.2 Adults

Recent analysis of NHANES–III data (between 1988 and 1994 and followed up to Dec 31, 2011) showed an exposure effect relationship between premature mortality and exposure to Pb (Lanphear et al., 2018; Landrigan et al., 2018). They aimed to quantify the relative contribution of environmental lead exposure to all–cause mortality, cardiovascular disease mortality, and ischaemic heart disease mortality, see Figure 3. Comparing mortality in the tenth percentile (BLL = 10 µg/L) with that in the 90th percentile (67 µg/L), they found the following hazard ratios [HR]:

- [HR] = 1.37 (95% CI: 1.17–1.60) in all–cause mortality;
- [HR] = 1.70 (95% CI: 1.30–2.22) in cardiovascular disease mortality;
- [HR] = 2.08 (95% CI: 1.52–2.85) in ischaemic heart disease.

A similar pattern of increased risk was seen when the analysis was restricted to people with BLL below 50 µg/L. Considering the exposure range, no effect under 10 µg/L could be calculated. Based on these ratios, population attributable fractions (PAF) could then be calculated and DALYs per year for selected health endpoints (i.e. premature mortality in adults due to exposure to lead). The methodology is based on calculation of proportion of participants that had BLL above 10 µg/L and calculation of BLL GM for those individuals.

For purpose of this task, DALYs calculations were performed only for all-cause mortality in adults above 20 years (ICD–10 code II to XVIII).

[11] To calculate the absolute burden, the absolute number of children (0-4y) in the sampling areas / regions was used. To compare this burden with other regions and internationally, the number per 100000 children was used.

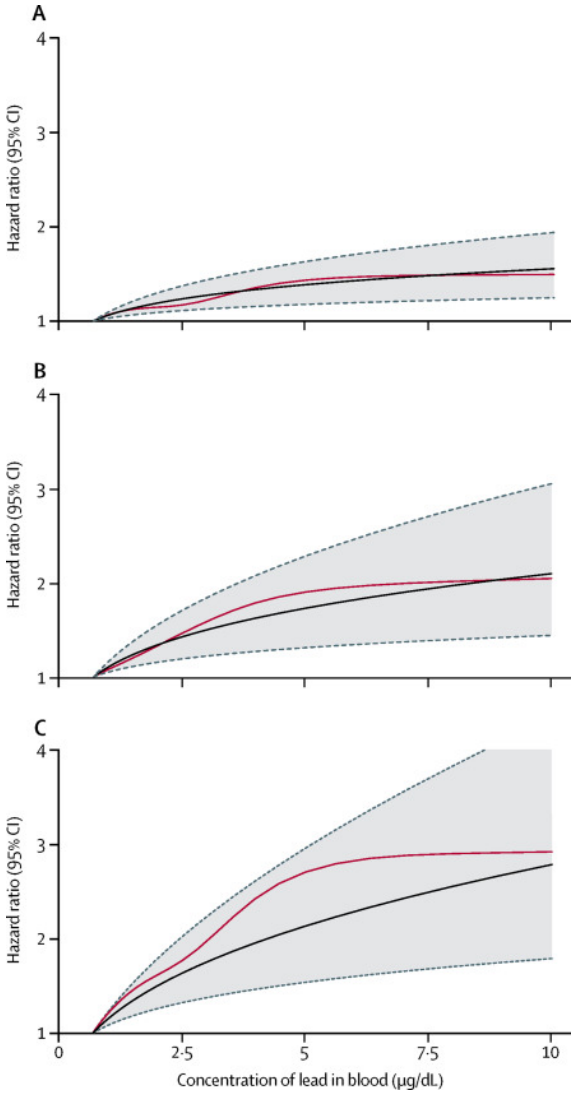

Figure 2: Dose–response curves for concentrations of lead in blood and mortality. Adjusted hazard ratios (black lines) with 95% CIs (hatched lines) and restricted cubic spline (red lines) for (A) all–cause mortality, (B) cardiovascular disease mortality, and (C) ischaemic heart disease mortality. Source: Lanphear et al., 2018.

Hazard ratio calculations, HR

Based on study Lanphear et al. (2018), hazard ratio in premature mortality (adults above 20 years) for increase of BLL from 10–67 µg/L (or 1,0−6,7 µg/dL) per log unit exposure (HR_{ue}) was calculated as follows:

$$HR_{ue} = 1 + (1{,}37 - 1) / (\log(6{,}7) - \log(1))$$

Then, HR at given log exposure (HR_{GM}) was calculated:

$$HR_{GM} = HR_{ue}\text{^}((\log(GM))$$

Where

GM = geometric mean of BLL in µg/dL of the adults with BLL above 1 µg/dL.

Population attributable fraction, PAF

Population attributable fraction was calculated according to the Levin equation:

$$PAF = f \times (RR - 1) / (f \times (RR - 1) + 1)$$

Where

f = a fraction of exposed population above 1 µg/dL (10 µg/L).

RR = HR_{GM} as calculated above.

Attributable number of cases per year, AC per year

$$AC_{per\ year} = N_m \times PAF$$

Where

N_m = number of deaths/year (all-cause mortality in this study) as obtained from the mortality statistics.

DALY / year

$$DALY_{per\ year} = AC \times 2,9$$

where

YLL = the potential years of life lost (YLL) for all-cause mortality. In Slovenia, average person dies around 3 years (2.9) [12] before his life expectancy, consequently.

6.4 Hazard assessment

In general, there is a lot of evidences for health effects at low levels of Pb in (bellow 50 µg/L) and no threshold level was identified for any of them. 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 BLL in children and adults (Wilhelm et al, 2010). 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: 40 µg/L for adult men, 30 µg/L for adult women and 35 µg/L for children (UBA, 2018; from the Scoping document on Pb).

In the past, the attention of lead in children was focused on BLL values ≥100 µg/L, however, recent evidence suggested that BLLs between 20 and 100 µg/L have been found to cause permanent cognitive impairment (Bellinger 2008a, 2008b; Binns et al. 2007; Lanphear et al. 2005). For mild mental retardation based on the WHO strategy (Fewtrell et al., 2003; Lanphear et al., 2005) an increase in BLL from 24 to 100 µg/L was associated with a decrement of 3.9 FSIQ (Full Scale IQ points (i.e., a slope of -0.051 points/µg/L (95% CI 0.032 to 0.070)), however, the values presented by the Gould (2009) were usually used, as presented in section 2.2.1. Often a threshold of 20 µg/L was used below which no effect was calculated. In several EU countries the impact of Pb on IQ in the EBoD studies was calculated following this strategy (Hänninen et al., 2014).

Later in life, in adults, Pb exposure causes adverse health effects, such as neurological disorders, hypertension, heart disease, stroke, kidney failure, high blood pressure, and osteoporosis (Abadin et al., 2007). Many of these conditions are chronic diseases that need to be managed over a lifetime, either with expensive drugs or with constant medical intervention.

[12] http://appsso.eurostat.ec.europa.eu/nui/show.do?dataset=hlth_cd_apyll&lang=en

6.5 Exposure assessment using Human Biomonitoring data

6.5.1 Children

To summarise data of above listed HBM studies in Slovenian children, BLL GM was slightly higher in DEMOCOPHES study (year 2011–2012; GM 12.71 µg/L) and in 59% of children from the Savinja region (14.53 µg/L) compared to 41% of children from the Central region (10.61 µg/L).

In CROME study (2016) BLL GM was 9.71 µg/L, and was lower in 90% of children from the Central region, comparing to 10% of children from some other regions, 9.54 µg/L versus 11.05 µg/L.

In the national HBM-II in the Mura region (2018) BLL GM was 8.43 µg/L and it was slightly higher in the age group 7–10 years comparing to age group 12–15 years, 8.88 µg/L and 7.81 µg/L, respectively. It seems that with the years BLL GM decreased slightly, despite children from different regions and of different age were considered. Assuming that the exposure of children in these years was similar, BLL GM was slightly higher in younger children and in older studies. This was expected to some extend due to more pronounced hand–mouth behaviour of younger children and constant reducing of exposure to Pb in recent years, however differences in studies were small.

The situation, however, was quite different in the Meža valley. As it was as already mentioned, the Meža valley is a highly polluted environment due to lead mine and smelter (see Introduction section). First measurements of BLL in 47 small children at 3 years of age from this area showed that more than one third of the children had BLL above 100 µg/L, and children from the UMV[13] having significantly higher BLL (median and average) as compared to children from the LMV[14] ($p<0.001$) (Eržen and Janet, 2005). Since 2004 systematically blood biomonitoring is performed annually in children 3 years of age in UMV. In 2007 the remediation measures were implemented to improve the quality of the environment (program is updated annually, based on CDC level of concern and proven feasible through remedial measures in the environment, as reported by von Lindern et al., 2016). First positive impacts of these measures were observed in the period 2004–2010 in decreased BLL in children. Since then BLL is still decreasing with a slight fluctuation within the years. Data from 2018 showed that 16.1% of children (3 years of age) had BLL between 50 and 100 µg/L and 50.6% of children had BLL below 50 µg/L. In 2019 and 2020 the situation improved even more as proportion of children with high BLL (above 50 µg/L) is decreasing (from the annual remediation program) and it was the lowest since the implementation of the measures in 2007.

6.5.2 Adults

To summarise data on the first national HBM-I study in 1084 participants (2008–2014), BLL GM was 18.4 µg/L and it was significantly higher in the Meža valley than in the other study areas (GM=27.2 µg/L versus 17.4 µg/L; $p<0.001$). In males, higher values were found than in females, GM 19.3 and 16.7 µg/L, respectively ($p<0.001$). This is consistent with the idea that males are thought to have higher BLL than female in general because of higher Pb exposure and blood hematocrit (Vahter et al., 2007). Moreover, premenopausal female release bone Pb more slowly than male, indicating a gender–specific discrepancy in Pb metabolism (Popovic et al., 2005).

Differences in BLL GM were significant between both parts of the Meža valley; UMV and LMV, 39.9 µg/L and 23.5. µg/L, respectively. The highest BLL GM values were found in male in UMV, 41.9 µg/L vs 26.0 µg/L in LMV. In two male BLL exceeded the population–based reference value of 90 µg/L in the blood as set for German population (Schulz et al., 2011) that was used for

[13] The Upper Meža river valley
[14] The Lower Meža river valley

comparison. In the lack of international consensus for population groups of increased susceptibility (pregnancy) and with reference to the guidelines regarding the screening and management of pregnant and lactating female in the US (Committe on Obstetric Practice, 2012) it was assessed, that six female in the study group exceeded the blood Pb level of 50 µg/L (three from UMV, the Upper Carniola and the Central Sava region, and three from the Central Slovenia region; i.e. 1% of the study population, similarly to the US population (IJS, 2015; Snoj Tratnik et al., 2019).

6.6 Results of EBoD calculations and discussion

6.6.1 Children

The results of EBoD estimation are presented in Table 1. In this Table, calculations based on BLL GM for children with BLL above 20 µg/L, and the sensitivity analyses where also children with BLL below 20 µg/L (all ranges BLL) are shown. In each region, total number of children (0–4 years) was considered, except in the Meža valley. In this case, only children living in this particular area were considered. This was due to the fact, that exposure was not similar across the region and because impact on children, traditionally exposed to Pb was seemed extremely important to be assessed (and stressed out), apart from the rest of this area. For better comparison, the numbers are presented per 100000 for better comparison.

The Meža HBM study related to two parts of the valley (UMV and LMV). The percentage of exposed children above 20 µg/L was the highest in 2–4 years old children from the UMV, i.e. 88.7% of children, with GM = 46.8 µg/L. Among them, 18 children (10.4%) were exposed to levels above 100 µg/L, with GM = 128.1 µg/L. One child had BLL at 209 µg/L. In the group of 77 children from the UMV, aged between 8 and 10 years, the percentage of exposed children above 20 µg/L was 88.3%, with GM = 40.5 µg/L. Among them, 2 children (2.6%) were exposed to levels above 100 µg/L (110 µg/L and 142 µg/L, respectively). In 200 children 2–4 years of age from the LMV, the percentage of children exposed to BLL above 20 µg/L was 52.5%, with GM = 33.5 µg/L. Comparing LMV children with those from UMV the difference was significant. However, one child from LMV had BLL well above 100 µg/L at 336 µg/L. Comparing the most recent data in the Mura region in 2018 (study 3) with studies 1 and 2, it could be observed that small % of children in the Mura had BLL above 20 µg/L; 2% versus 11 and 4% in studies 1 and 2, respectively, and none of children in these studies had BLL above 100 µg/L.

Results in terms of DALYs (per 100000 children), the values were estimated at 18 (95% CI: 11−26) in CROME in children 7−8 years, at 20 (95% CI: 13−20) in DEMOCOPHES in children 6−12 years, and at 24 (95% CI: 15−33) and 26 (95% CI: 16−37) in the national HBM-II study in children 12−15 years and 7−10 years, respectively.

Apart from this, DALYs were estimated significantly higher in the Meža valley, especially in the UMV, where DALYs were at 3437 (95% CI: 2088−4955) in children 2−4 years in the UMV, following by DALYs at 2556 (95%CI: 1566−3653) in children 8−10 years in the UMV, while in children 2−4 years in the LMV, DALYs were 978 (95% CI: 605−1384). Those numbers were more than 10-fold higher than in children, participating in other SLO HBM surveys, listed above.

Based on the sensitivity analysis, the number of DALYs (per 100000) was the lowest in group of adolescents in the Mura region (study 3), followed by the group of children aged 7-10 years in that region, and then by the groups involved in DEMOCOPHES and CROME surveys (studies 1 and 2). These results were just opposite, when using BLL above 20 µg/L, calculating lower impact in the studies 1 and 2. This could be explained by the fact, that all ranges BLL GM was higher in the studies 1 and 2 compared to the Mura study, probably because of very few children with more pronounced elevated Pb exposure in this region. However, these estimates should only be compared with caution, because of different time periods and differences in age groups of children,

involved in these studies. Nevertheless, by assuming that studies 1 and 2 represented the same region (Central region) and that exposure was similar overall in the Central region, it could be summarised, that impact decreased from 2011 to 2016 in this region.

In Table 2, EBoD estimates in children, based on BLL data in selected EU countries as provided from the HBM4EU repository (available through IPCHEM) are shown. In Belgium (Flanders) based on FLEHS IV study in 2016−2020, none of adolescents of 14−15 years had BLL above 20 µg/L. It could be observed from the FLEHS studies (from 2003 and on), that the average BLL among young people from the general Flemish population decreased from 22,4 µg/L in FLEHS I to 14,6 µg/L in FLEHS II, then to 9,4 µg/L in FLEHS III and to 7,8 µg/L in FLEHS IV ($p<0.001$ for each decrease). The BLL GM among Flemish young people have decreased by 65 % over the past 15 years (https://www.milieu-en-gezondheid.be/sites/default/files/atoms/files/Referentierapport__versie3_juni2021_0.pdf). In Czech R., based on CzechHBM-CE study in 2016−2017, 17% of children 3−5 years and 5% of children 6−11 years had BLL above 20 µg/L, with BLL GM at 26 and 23 µg/L, respectively. In Germany, based on GerES V study in 2014−2017, BLL above 20 µg/L was found in 7% of children 3−5 years with BLL GM at 25 µg/L; then in 6% of children 6−10 years with BLL GM at 23 µg/L; in 3% of adolescents 11−13 years with BLL GM at 22 µg/L, and in 3% of adolescents 14−17 years with BLL GM at 23 µg/L. Corresponding DALYs (per 100000 children), in Czech Republic DALYs amount to 127 (95%CI: 80−178) and 20 (95%CI: 12−28) in two age groups, respectively. In Germany, DALYs were 48 (95%CI: 30−68), 24 (95%CI: 15−34), 7 (95%CI: 5−10) and 11 (95%CI: 7−16) in four age groups, respectively. Considering sensitivity analysis in children in selected EU countries, DALYs (per 100000 children), were as follows: in Czech R. 1787 (95%CI: 1106−2528) and 1601 (95%CI: 992−2260) in two age groups, respectively; in Germany, 1417 (95%CI: 880−1996), 1445 (95%CI: 897−2037), 1136 (95%CI: 707−1596) and 1150 (95%CI: 716−1616) in four age groups, respectively, and in Belgium, 1039 (95%CI: 647−1458).

In general, DALYs were higher in younger children. As mentioned already above, comparison of these results is not fair, at least due to different time periods in which these studies were performed. However, among the children in the selected EU countries, the burden is the highest for Czechs children, aged 3−5 years, followed by their pears from the Germany. These two studies could be compare with certain credibility also with the Slovenia studies 2, 3 and 4, at least in terms of time period of their performance. While comparing the results of EBoD calculations, including the sensitivity analysis for Slovenian children and adolescents to their pears from the selected EU countries, it is observed, that burden of disease is the highest in the spot area of the Meža valley. Finaly, it could be summarised, that DALYs in Slovenian children, based on the most recent HBM SLO data, excluding the Meža valley, were similar to those in selected EU countries from various time periods.

6.6.2 Adults

The results of EBoD estimation for Slovenia are shown in Table 3. Participants from twelve statistical regions were combined in ten study groups, representing the Meža valley (Carinthia region), as a hot spot (see section 2.1.2 and Figure 1). Overall Slovenian data (with and without the Meža valley) and differences in both genders are also shown. Mortality and population data (over 20 years of age) were taken from official statistics for each statistical region (2013 data). In the Meža valley these numbers represented only the area where sampling took place and not the entire Carinthia region. This was due to the fact already explained in the former section 5.1. For better comparison between the regions / groups and internationally, the numbers per 100000 are presented.

In the Meža valley, 98% of participants had BLL above 10 µg/L with GM at 28 µg/L. This was significantly higher than in different individual groups of adults in the HBM-I SLO and overall HBM SLO, with BLL GM in the range 17−20 µg/L and 19 µg/L, respectively. Corresponding DALYs (per 100000) were the highest in the Meža valley, 464 (95%CI: 244-661) versus 311 (95% CI: 161−450) in Slovenia overall. Considering differences between genders, DALYs were higher in male comparing to female, 348 (95%CI: 181−502) and 276 (95%CI: 142−399), respectively.

For comparison, EBoD estimates were performed in adults in selected EU countries as provided from the HBM4EU repository (available through IPCHEM). The results are shown in Table 4. In Belgium, based on FLEHS 1 study in 2004−2005, 100% of participants 40−59 years had BLL above 10 µg/L, with BLL GM at 37 µg/L. This is at the same point the oldest HBM data, approximately 5−10 years older than others. The most recent available data were those from Czech Republic HBM in 2015, in two groups of adults, 20−39 years and 40−59 years. It was observed that 75% of younger participants had BLL above 10 µg/L with BLL GM at 19 µg/L, while 86% in the elder group, had BLL above 10 µg/L with BLL GM at 23 µg/L. In Spanish HBM in 2009−2010, 97% of participants between 20−39 years had BLL above 10 µg/L with BLL GM at 21 µg/L, and 99% of participants between 40−59 years had BLL above 10 µg/L with BLL GM at 28 µg/L. The corresponding DALYs were 733 (95%CI: 390−1031) in Belgium, in Czech R. at 386 (95%CI: 198−562) and 561 (95%CI: 290−809), considering both age groups, and in Spain at 310 (95%CI: 161−445) and 428 (95%CI: 225−608), considering both age groups.

Again, comparison of these results is not fair due to different time periods in which these studies were performed. In general, DALYs were calculated higher based on older data sets, e.g. in Belgium FLEHS I study (2004-2005) comparing to Czech R. data from 2015. It could be noted, that a high proportion of adults in these studies had BLL higher than 10 µg/L and that BLL GM of these persons was still around 20 µg/L. However, regarding old HBM data on Pb, it would be worthy to perform new survey to estimate reliable and current impacts at EU level. It aslo should be noted the uncertainties related to the dose-responses at low exposure levels. Lanphear et al 2018 study is anyway the first one suggesting increased mortality at the levels <70 µg/L and confounders cannot be totally excluded. As discussed by the authors, one uncertainty is related to the fact that exposure assessment was based on single BLL measurement. Therefore, there are also some uncertainties related to this dose response and more studies are also needed to confirm this.

Table 1: EBoD calculations for children from different SLO HBM studies per 100000 and sensitivity analysis

No.	Year of study	Study / Location / Group	Sample size / age (years)	Children[1], n (0–4 yrs)	% above 20 µg/L	GM, µg/L (BLL >20 µg/L)	GM, µg/L (all ranges BLL)	DALY* per 100000, (95% CI)	DALY** per 100000, (95% CI)
1	2011–2012	DEMOCOPHES Central region	95 / 6–12	28952	11	22	13	20 (13–28)	1754 (1086–2480)
2	2016	CROME Central region	135 / 7–8	28952	4	24	10	18 (11–26)	1301 (809–1831)
3	2018	HBM-II Mura region	135 / 7–10	4956	2	33	9	26 (16–37)	1203 (749–1692)
		HBM-II Mura region	94 / 12–15	4956	2	29	8	24 (15–33)	1055 (658–1482)
4	2018−2020	THE MEŽA HBM UMV	77 / 8–10	1125	88	41	36	2556 (1566–3653)	5366 (3221–7834)
		THE MEŽA HBM UMV	195 / 2–4	1125	89	47	42	3437 (2088–4955)	6326 (3769–9307)
		THE MEŽA HBM LMV	200 / 2–4	1125	53	34	22	978 (605–1384)	3182 (1945–4560)

[1]: For the Meža valley, only children living in the area, were considered. *:DALYs for children having a BLL higher than 20 µg/L. **DALYs for children for all ranges of BLL (sensitivity analysis). 95% CI = 95% confidence interval. UMV – Upper Meža valey. LMV – Lower Meža valey.

Table 2: EBoD calculations for children selected EU countries per 100000 and sensitivity analysis

No.	Year of study	Country / Study	Sample size / age (years)	Children (0–4 yrs)	% above 20 µg/L	GM, µg/L (BLL >20 µg/L)	GM, µg/L (all ranges BLL)	DALY* per 100000, (95% CI)	DALY** per 100000, (95% CI)
1	2016−2017	Czech Republic / CzechHBM-CE	159 / 3−5	568823	17	26	13	127 (80−178)	1787 (1106−2528)
2	2016−2017	Czech Republic / CzechHBM-CE	252 / 6−11	568823	5	23	12	20 (12−28)	1601 (992−2260)
3	2014−2017	Germany / GerES V	138 / 3−5	3961376	7	25	10	48 (30−68)	1417 (880−1996)
4	2014−2017	Germany / GerES V	231 / 6−10	3961376	6	23	11	24 (15−34)	1445 (897−2037)
	2014−2017	Germany / GerES V	143 / 11−13	3961376	3	22	8	7 (5−10)	1136 (707−1596)
	2014−2017	Germany / GerES V	208 / 14−17	3961376	3	23	9	11 (7−16)	1150 (716−1616)
5	2016−2020	Belgium / FLEHS IV	419 / 14−15	609407	0	/	8	/	1039 (647−1458)

*:DALYs for children having a BLL higher than 20 µg/L. **DALYs for children for all ranges of BLL (sensitivity analysis).

Table 3: All–cause mortality DALYs per 100000 (HBM SLO data, 2008−2014, age 18−49),

Group no.	Location (region) Sex	Sample size	Population, n (>20 years)	% above 10 µg/L	GM, µg/L (BLL >10 µg/L)	Deaths per year (>20 yrs)	RR	PAF	DALY per 100000 (95% CI)
1	CENTRAL+INN.CARNIOLA	114	475290	94	19	4724	1,11	0,09	267 (138−385)
2	SOUTHEAST	158	170287	92	20	2118	1,12	0,10	364 (188−525)
3	SAVINJA+LOWER SAVA	160	209216	93	19	2453	1,11	0,09	308 (159−445)
4	GORIZIA	98	96571	82	18	1259	1,10	0,08	286 (147−415)
5	COASTAL–KARST	100	92868	98	18	1018	1,10	0,09	279 (144−403)
6	UPPER CARRNIOLA	83	162154	87	19	1737	1,11	0,09	266 (137−385)
7	DRAVA	100	265307	92	19	3345	1,11	0,09	335 (173−484)
8	MURA	88	96783	89	17	1363	1,09	0,07	302 (155−438)
9	CENTRAL SAVA	104	35736	95	18	511	1,10	0,09	357 (185−517)
10	MEŽA VALLEY	79	20785	98	28	223	1,18	0,15	464 (244−661)
	SLOVENIA total	1084	1624997	92	19	18751	1,11	0,09	311 (161−450)
	Men	549	796871	92	21	9248	1,13	0,10	348 (181−502)
	Women	535	828126	92	18	9503	1,10	0,08	276 (142−399)
	SLO. total excl. MEŽA	1005	1604212	91	19	18528	1,11	0,09	296 (153−428)
	Men	500	786478	91	20	7946	1,12	0,10	282 (146−408)
	Women	505	817734	92	18	9398	1,10	0,08	268 (138−388)

Note: CI = 95% confidence interval, YLL per case = 2,9

Table 4: All–cause mortality DALYs per 100000, selected EU countries

Country / Study	Year of study	Sample size / age (years)	Population , (>20yrs)	% BBL > 10 µg/L	GM, µg/L BLL >10 µg/L	Deaths per year (>20yrs)	RR	PAF	YLL	DALY per 100000 (95% CI)
Spain / BIOAMBIENT.ES	2009-2010	1121 / 20−39	38055468	97	21	414214	1,13	0,11	2,6	310 (161−445)
Spain / BIOAMBIENT.ES	2009-2010	709 / 40−59	38055468	99	28	414214	1,18	0,15	2,6	428 (225−608)
Czech Republic/ CzechHBM-AE	2015	140 / 20−39	8505707	75	19	111751	1,11	0,08	3,9	386 (198−562)
Czech Republic/ CzechHBM-AE	2015	148 / 40−59	8505707	86	23	111751	1,14	0,11	3,9	561 (290−809)
Belgium / FLEHS 1	2004−2005	980 / 40−59	8943884	100	37	108047	1,24	0,19	3,2	733 (390−1031)

Note: Aggregated data (50th and 90th percentiles; P50 and P90) was used from the HBM4EU repository (available through IPCHEM) and based on a log-normal fit, the percentage of the subjects with more than 10 and 20 µg/L lead in blood was calculated

6.7 Conclusions

The environmental burden of disease (EBoD) was calculated in children and adults, based on Pb levels in blood obtained in Human Biomonitoring surveys. For these reason, different available data of HBM studies in SLO were considered, inculding different regions, age groups and time period. In addition, this data was compared with selected HBM studies from the HBM4EU repository, including Spain, Czech Republic, Germany and Belgium. The EBoD was calculated as DALYs for developmental neurotoxicity in young children and adolescents (lost cognitive development) and premature mortality (all–cause mortality) in adults.

The proportion of children having blood lead levels higher than 20 µg/L was the highest in Slovenian children from the Meža valley, one of the most polluted regions in the EU and world-wide also. Consequently the calculated DALYs were the highest in this Slovenian region. When excluding these children from the EBoD calculations, DALYs were then comparable to those from selected EU countries. In general, DALYs were higher in younger children and in studies of older date. However, comparison of these estimates should only be compared with caution, because of different time periods, differences in age groups of participants, and other potential differences, that could not be considered. It could be pointed out that beside the Meža valley hot spot, there are more of such hot spots in the EU which might need more attention in further assessments.

In adults, DALYs were calculated higher in older data sets, e.g. in Belgium FLEHS 1 study (2004-2005) comparing to Czech R. data from 2015. It could be summarised, that a high proportion of adults in these studies had blood lead levels higher than 10 µg/L with geometric mean around 20 µg/L. However, DALYs for premature deaths relating to Pb exposure could not be directly compared, since HBM data used was old. It would be worthy to perform new survey to estimate reliable and current impacts at EU level.

There are also some uncertainties related to the dose-responses at low exposure levels; especially in the case of adult's mortality, suggesting increased mortality at the levels <70 µg/L and confounders cannot be totally excluded. In addition, the uncertainty is related also to the fact that exposure assessment was based on single BLL measurement, and more studies are needed to confirm this. The results in this report seemed not only to be useful as overview of the situation in EU, but also in achieving one of the common goals of HBM4EU, e.g., harmonisation of the HBM protocols and consequently better understanding and comparison of human exposure and health outcomes. However, new HBM data at the EU level are highly recommended to get an overview on the current situation in the EU, as well as for a more reliable comparison and up to date health impact assessment.

6.7.1 Conclusions in the context of policy questions

PQ1. What is the concentration of lead in the human blood nowadays (after phasing out leaded petrol) in the countries of Europe?

The highest proportion of children, having BLL above 20 µg/L, was identified in Slovenia, in the Upper Meža valley hot−spot, where 89% of children, aged 2−4 years had BLL GM = 47 µg/L, followed by 88% children, aged 8−10 years from the same area, with BLL GM = 41 µg/L. In addition, 10% of children in the Upper Meža valley had BLL above 100 µg/L and some of them also above 200 µg/L. In other SLO HBM studies, a small percentage (2−14%) had BLL above 20 µg/L with BLL GM between 22−33 µg/L, and none of children had BLL above 100 µg/L. In a recent Belgian HBM study (FLEHS IV), 0% of adolescents of 14−15 years had BLL above 20 µg/L. In the Czech Republic (CzechHBM−CE_2016), 5−17% of children had BLL above 20 µg/L, with BLL GM at 23 and 26 µg/L in age groups 6−11 and 3−5 years, respectively. In Germany (GerES V,

2014−2017), the proportions of children with BLL above 20 µg/L were as follows: 7% in the age group 3−5 years, 6% in the age group 6−10 years, 3% in the age group 11−13 years, and 3% in the age group 14−17 years. The corresponding BLL GMs were 25, 23, 22 and 25 µg/L, respectively.

In adults, the highest proportion of participants with BLL above 10 µg/L were identified in Belgium FLEHS I study, were 100% of participants of 40−59 years had BLL above 10 µg/L with BLL GM at 37 µg/L. This was followed in Slovenian adults, in the Meža valley, where 98% of participants of 18−49 years had BLL above 10 µg/L, with BLL GM at 28 µg/L. In Slovenian total population, this proportion was 92%, with BLL GM at 19 µg/L, and it was higher in men than in women, 21 and 18 µg/L, respectively. In two age groups of adults in Spanish HBM, 97% of participants of 20−39 years had BLL above 10 µg/L with BLL GM at 21 µg/L, and 99% of participants of 40−59 years had BLL above 10 µg/L with BLL GM at 28 µg/L. In two age groups of Czech's adults, 75% of participants of 20−39 years had BLL above 10 µg/L with BLL GM at 19 µg/L, while in the elder group of 40−59 years, 86% had BLL above 10 µg/L with BLL GM at 23 µg/L.

The BLL still indicate exposure to lead in children especially in a part of Slovenia due to former industrial activities with lead. In adults, based on the most recent HBM data from Czech Republic (2016), exposure to lead is still indicated.

PQ2. What is the EBoD due to Pb exposure in children and adults, based on available HBM data (and potential comparison with other – selected– countries).

The environmental burden of disease (EBoD) was expressed as disability-adjusted life years (DALYs) per 100,000 people.

In children, corresponding DALYs were the highest in Slovenia in the Meža valley in general, 978−3437 (95%CI: 605−4955) per 100,000 children, which was more than 10−fold higher than in other Slovenian regions in general, 18−26 (95% CI: 11−37). This was also significantly higher than in children in selected other EU countries. In the Czech Republic, DALYs were 19 and 127 (considering two age groups), and between 8 and 47 in Germany (considering four age groups). In general, DALYs were higher in younger children of 3−5 years, comparing children of age between 6 and 10 years and adolescents of 11-17 years. It is also obvious from numerous studies that over time BLLs have decreased significantly (e.g. FLEHS IV and GerES V).

In adults, corresponding DALYs were 464 (95%CI: 244−661) per 100,000 adults in Slovenia in the Meža valley versus 311 (95% CI: 161−450) in Slovenia overall. Regarding gender, DALYs were 348 (95%CI: 181−502) and 276 (95%CI: 142−399) in men and women, respectively. Based on FLEHS I study in Belgium, corresponding DALYs were calculated at 733. In two age groups of Spanish HBM, DALYs were 314 and 427, respectively. In two age groups of Czech's adults, DALYs were 385 and 546, respectively. In general, DALYs were calculated higher in elder adults, and based on an older date HBM surveys (Belgium).

PQ3. Do blood lead levels of both adults (especially pregnant women) and children still indicate permanent existence of lead exposure and what kind of exposure sources are the most important?

Blood lead levels in children and pregnant women still indicated permanent lead exposure in Slovenia, especially in the Meža valley, one of the most polluted regions in the EU and world-wide. Consequently, the calculated DALYs were the highest in this Slovenian region. When excluding these children from the comparison with children from the other EU countries, the values were comparable.

Based on the most recent HBM data from Belgium (FLEHS IV), none of children had BLL above 20 µg/L, while data from Germany (GerES V) indicated only a small proportion of children that had

BLL above 20 µg/L with geometric mean up to 25 µg/L. In the Czech HBM cohort (CzechHBM-CE), a higher proportion of children than in Germany had a BLL above 20 µg/L, with a geometric mean around 24 µg/L. However, this may still indicate permanent existence of lead exposure.

In adults, BLLs were higher in older data sets, e.g. in Belgium FLEHS 1 study (2004-2005) comparing to the CzechHBM-AE data from 2015. However, in the most recent data from Czech HBM, 75 to 86% of adults in two age groups had BLL higher than 10 µg/L with geometric mean around 20 µg/L, which may indicate potential permanent existence of lead exposure in European population. However, DALYs for premature deaths relating to Pb exposure could not be directly compared, since HBM data used was old. It would be worthy to perform a new survey to estimate reliable and current impacts at EU level.

Gathering of new HBM data at the EU level is highly recommended to get an overview on the current situation in the EU, as well as up to date data for a more reliable comparison and health impact assessment.

The blood lead levels still indicate exposure to lead in children especially in a part of Slovenia due to former industrial activities with lead. In adults, based on the most recent HBM data from Czech Republic (2016), exposure to lead is still indicated also.

6.8 References

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7 Mycotoxins full RA report

Risk assessment calculation for mycotoxins – the case-study of deoxynivalenol

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Acknowledgements

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7.1 Summary

Mycotoxins are a group of chemical food contaminants, including aflatoxins, deoxynivalenol, zearalenone, patulin, fumonisins, among others. These compounds are secondary metabolites of fungi that contaminate food commodities at different stages of the food chain, namely in the production, harvest, storage, and processing. Mycotoxins are relevant in a public health perspective due to their potential to have several toxic effects in humans such as carcinogenic, genotoxic, teratogenic, estrogenic, and immunomodulatory effects. After a prioritisation process, deoxynivalenol and fumonisin B_1 were included in the 2nd set of substances prioritised under HBM4EU. Fumonisin B_1 was not included in the present report because there is very limited Human Biomonitoring data to support its exposure assessment. This is due to its low urinary recovery and high inter-individual variability in absorption and excretion, which leads to a high uncertainty, not allowing an accurate risk characterisation. As such, this report pertains only to deoxynivalenol (DON).

The general aim of this report is to assess the risk associated to human exposure to DON, in populations from different European countries or regions based both on published Human Biomonitoring data and on the new data generated for the adult population in the context of the aligned studies developed across Europe (NIOM_POLAES, UI_DIET_HBM, UBA_ESB and LNS_Oriscav-Lux2 studies). For both databases, two approaches were followed, *i*) using Human Biomonitoring data and estimating the external exposure (Probable Daily Intake, PDI) through reverse dosimetry and thereafter, determining the hazard quotient through comparison with the available external health-based guidance value, and *ii*) comparing the Human Biomonitoring data with the Human Biomonitoring guidance value (HBM-GV) determined for DON in the scope of the work developed under Task 5.2. Results for risk characterisation regarding data obtained in aligned studies will be further detailed in a future publication under HBM4EU [Namorado et al. (in preparation); provisional title: “Current exposure of the European adult population to mycotoxins: results from the HBM4EU aligned studies”].

The results showed that exposure to DON in the European population is generalised, affecting different age groups of the population. Children and pregnant women, which are traditionally considered vulnerable population groups, presented the highest risk. The children group deserves particular attention considering the associated vulnerability and the potential long-term consequences that are difficult to predict. The Human Biomonitoring guidance value for DON derived under task 5.2 of the HBM4EU project allowed for the first time, to assess the risk of DON based on exposure biomarkers and an HBM-GV. Results from the aligned study conducted in adult population from Poland showed that the highest percentiles of exposure (P90 and P95) represented a potential health concern since the hazard quotient determined is above one. However, the mean and median levels of exposure were considered as not representing a concern for health. Results obtained from the aligned studies conducted in Iceland, Germany and Luxembourg revealed an exposure to DON that does not represent a health concern.

The use of HBM data for mycotoxins implies an extensive knowledge of metabolism but there are still some gaps regarding mycotoxins’ toxicokinetic data that may hamper a proper risk assessment. If developing a risk assessment for regulatory purposes, all these aspects are important to be considered and assume a higher relevance. The establishment of an HBM-GV is of major relevance for performing a more accurate risk characterisation, allowing a direct comparison of exposure with a reference value, and reducing the uncertainty in estimates. However, regarding compounds for which a reduced knowledge on metabolism is available, the issue of uncertainty in estimates remains and the limitations of the HBM-GV should be described in detail.

Considering the present report, the use of HBM data for risk assessment encompasses some limitations that are related with the use of data published in several scientific articles, non-harmonised sample collection and criteria for left-censored data. These limitations could be overcome in a near future by developing of guidance for setting-up biomonitoring campaigns that would allow a proper comparison of exposure among studies whole lowering the uncertainties that affect risk assessment.

7.2 Introduction

Mycotoxins are secondary fungal metabolites often found as natural contaminants in agricultural commodities all over the world and their occurrence poses a risk to human and animal health (1,2). Indeed, mycotoxins have the potential to produce several toxic effects in humans such as carcinogenic, genotoxic, teratogenic, estrogenic, and immunomodulatory effects. After a prioritisation process, deoxynivalenol and fumonisin B_1 were included in the 2nd set of substances prioritised under HBM4EU. Fumonisin B_1 was not included in the present report because there is very limited Human Biomonitoring data to support its risk assessment. This is due to its low urinary recovery and high inter-individual variability in absorption and excretion, which leads to a high uncertainty, not allowing an accurate risk characterisation. As such, this report pertains only to deoxynivalenol (DON).

DON is a mycotoxin produced by *Fusarium* species (mainly *Fusarium graminearum* and *Fusarium culmorum*). These fungi that grow on cereals in the field in areas with temperate climates, are commonly found in Europe (3). Frequently known as “vomitoxin” due to its acute effects at the gastrointestinal tract (2), DON is classified by IARC in group 3 meaning that there is no evidence of carcinogenicity to humans (4). DON has been identified as one of the mycotoxins that occurs widely in cereal products (wheat, barley, oats, rye and maize) (6,7,8) and it has been referred that exposure may exceed the TDI of 1 µg/kg bw/day (6). Besides the acute effects, human exposure to DON is associated with chronic health effects that cannot be neglected such as altered nutritional efficiency, weight loss, and anorexia. The main toxicity mechanism has been described as the capacity to bind to eukaryotic ribosomes and inhibit protein synthesis (4). In 2017, EFSA considered appropriate to include the acetylated and modified forms (3-ADON, 15-ADON and DON-3G) in the assessment performed, thus considering a group TDI of 1 µg/kg bw/day for DON based on reduced body weight gain in mice as the critical chronic effect for human risk assessment (3).

Under the HBM4EU initiative, several policy-related questions were identified. Among these, two main policy-related questions concerning risk assessment should be highlighted:

- Is the risk associated to human exposure to these mycotoxins characterised?

Is it possible to set a HBM guidance value (HBM-GV) for mycotoxins?

The general aim of this report is to assess the risk associated to human exposure to DON, in populations from different countries or regions of the EU, based on Human Biomonitoring (HBM) published data and on the new data generated in the adult population in the context of the aligned studies developed.

7.3 Methodology

For the present report, two different approaches for risk assessment were followed considering the data available for DON:

- Using HBM data and estimating the external exposure (Probable Daily Intake, PDI) through reverse dosimetry and thereafter, determining the hazard quotient through comparison with the available external Health-Based Guidance Value (group TDI for DON: 1 µg/kg bw/day);
- Comparing the HBM data with the HBM-GV determined for DON in the scope of the work developed under Task 5.2 (Total DON: 0.69 µg DON/kg bw/total 24h ≈ 23 µg DON/L urine (Confidence Interval: 5-33 µg/L)) (7).
- HBM data on mycotoxins used for this risk assessment was obtained from bibliographic search, done in WP10.4 for DON and, in a second stage, through the development of HBM4EU aligned studies in different European regions (8).

In this study, risk assessment was performed mainly for the general population considering that human exposure to mycotoxins is mostly attributed to food contamination, and consequently affecting all population. However, since other exposure sources such as specific occupational environments (e.g., food and feed processing, animal production, waste management) are also possible, occupational exposure was also included when relevant data was available.

7.3.1 Data collection

7.3.1.1 Bibliographic search

A bibliographic search was performed using PubMed, Scopus, and Web of Science databases to identify HBM studies published between 2000 and 2020. The terms used for the search were “exposure” and “biomonitoring” and “mycotoxins”. EFSA reports were also considered to obtain more information on these topics. From the 306 articles gathered, 100 articles were compiled in a single database after duplicates exclusion and application of inclusion criteria (European population studies published between 2000 and 2020 reporting mycotoxins Human Biomonitoring data). The database included HBM data concerning mycotoxins and their metabolites such as: type of biological samples collected (urine, blood, and breast milk), mycotoxins concentration, number of samples analysed, % of positive samples, methods, and analytical conditions (limits of detection and quantification, % of recovery), geographic area, sampling years, number of participants, and available relevant demographic information (age, sex, urban/rural residence, etc). When possible, information of exposure determinants (including occupational exposure, indoor use, or dietary information) was also included in the database. For DON and its metabolites, 40 articles were identified (Annex 1).

7.3.1.2 Aligned studies

Under the HBM4EU project, aligned studies were developed aiming to characterise the exposure of the European population to several contaminants. These studies included the harmonisation of protocols for study design, sampling, and analysis under the work packages 8 and 9. For mycotoxins, first morning urine samples were collected from a sample representative of the national population. The data available refers to a sample of the adult population from Poland (n=193, study NIOM_POLAES), Iceland (n=171, study UI_DIET_HBM), Germany (n=120, study UBA_ESB) and Luxembourg (n=191, study LNS_Oriscav-Lux2). Risk characterisation results will be further detailed in a future publication under HBM4EU [Namorado et al. (in preparation); provisional title: “Current exposure of the European adult population to mycotoxins: results from the HBM4EU aligned studies”].

7.3.2 Probable Daily Intake and Risk Characterisation

The probable daily intake (PDI) for DON was determined through reverse dosimetry calculation in order to convert the urinary mycotoxin concentrations into intake levels, expressed as µg/kg bw/day (9,10). The deterministic method of intake mass balance was applied, considering the concentration of biomarker in urine (µg/L), the urinary volume (L), the body weight (kg), the excretion rate for DON (%) and the mass balance between the parent compound and the metabolite (11), according to the following formula:

$$\textbf{DON PDI (µg/kg bw/day)} = \frac{(uDON + ((uDON\text{-}15\text{-}GlcA + uDON\text{-}3\text{-}GlcA)*0.63) + (uDON\text{-}3G*0.64) + uDOM\text{-}1)* UV * 100}{BW * ER}$$

uDON = concentration of DON in urine (µg/L); uDON-15-GlcA = concentration of DON-15-GlcA in urine (µg/L). uDON-3-GlcA = concentration of DON-3-GlcA in urine (µg/L); uDON-3G = concentration of DON-3G in urine (µg/L). uDOM-1 = concentration of DOM-1 in urine (µg/L); 0.63 = Molar ratio for DON/DON-3-GlcA and DON/DON-15-GlcA. 0.64 = Molar ratio DON/DON-3G; UV = urinary volume in 24h (L); BW = body weight (kg); ER = excretion rate for DON = 64%

Regarding the reverse dosimetry calculations from the urinary biomarkers, and in the absence of data at individual level for all the studies considered, the following parameters were assumed, based on published work:

- Body weight for studies obtained in bibliographic search: adults (70 kg), adolescents (51 kg), children (23 kg) (12)
- Body weight for aligned studies: 80.3 Kg (Poland), 80.9 Kg (Iceland), 80.2 Kg (Germany), 78.0 Kg (Luxembourg) (13)
- Daily urinary volume: adults (2 L), adolescents (1 L), children (0.5 L) (12)
- Excretion rate for DON (64%) (14)

Risk characterisation was performed comparing the exposure determined for population with the respective health-based guidance value to determine the Hazard Quotient (HQ). When the HQ was < 1, the exposure was considered to be within safe limits (15). The HQ was calculated as follows:

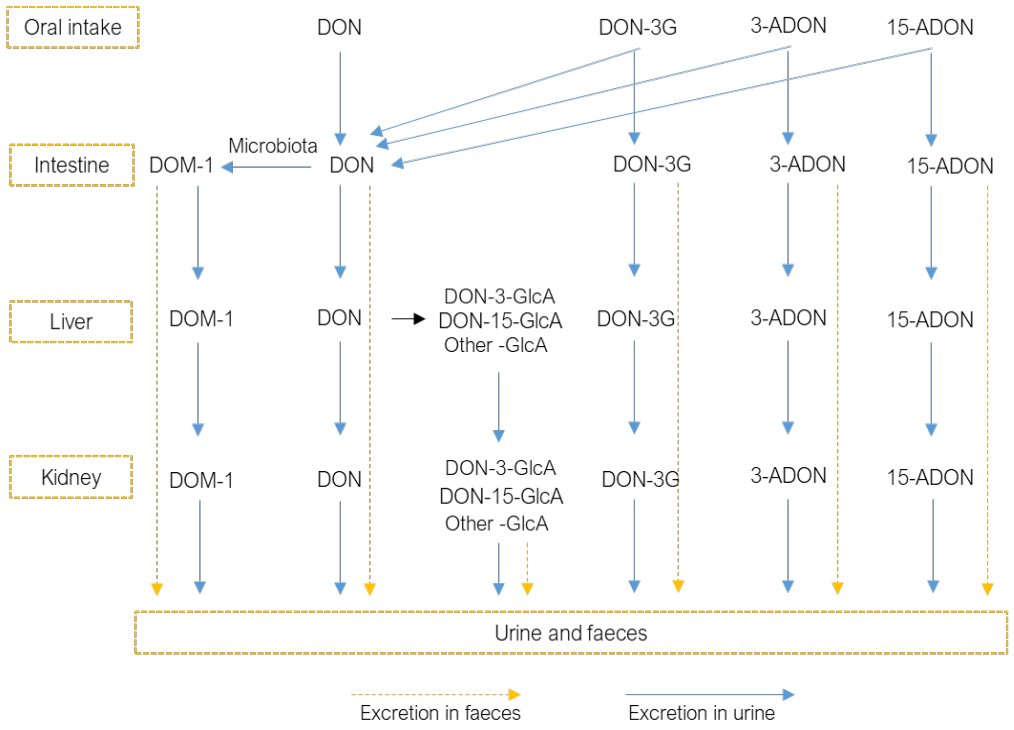
- Ratio between PDI and Tolerable Daily Intake (TDI) for DON (1.0 µg/kg bw/day)
- Ratio between urinary exposure biomarkers and HBM-GV for DON (23 µg/L)

7.4 DON metabolism relevant for hazard assessment

The present report considered toxicological data for DON already available in the literature. Besides inducing acute toxicity characterised by gastrointestinal symptoms like emesis, which inspired the designation as “vomitoxin” (2), DON is also immunotoxic and has been associated with developmental and reproductive toxicity. No carcinogenic effects have been reported. In animals and humans, prolonged exposure to DON reduces food intake and nutrient absorption and induces body weight loss. These effects are related to intestinal factors, such as hormone and pro-inflammatory cytokines (16).The molecular mechanisms that mediate DON effect on the immune system are not completely disclosed but seems to be associated with its capacity to activate mitogen-activated protein kinases (MAPKs) and induce apoptosis in a process known as the “ribotoxic stress response” (17) . Although in vitro and in vivo toxicity studies have generated some conflicting data, in general they suggest that the modified forms of DON; e.g, the acetylated forms display a toxicity similar to that of DON. They are also able to induce oxidative stress and trigger ribotoxic effects (18). As such, after exposure, the toxicity of those forms contributes to the overall DON toxicity.

Briefly, the data regarding DON metabolism relevant for this assessment is presented here. For DON, three main pathways of DON are described (19,20):

1. the biotransformation by intestinal or ruminal microbes to de-epoxy-deoxynivalenol (DOM-1) that represents an important metabolic pathway in ruminants, but with moderate importance in pigs and humans.
2. the conjugation with glucuronic acid resulting in the formation of deoxynivalenol-glucuronide (DON-GlcA), whose production rates differ considerably among species.
3. the sulfonation with sulfonate resulting in the formation of deoxynivalenol-sulfonate, a pathway identified in chickens and rats (19,20)



DON: deoxynivalenol; DON-3G: deoxynivalenol-3-glucoside; 3-ADON: 3-acetyl-deoxynivalenol; 15-ADON: 15-acetyl-deoxynivalenol; DOM-1: deepoxy-deoxynivalenol; DON-3-GlcA: deoxynivalenol-3-glucuronide; DON-15-GlcA: deoxynivalenol-15-glucuronide

Figure 4: Human metabolism of DON (21)

The Figure 1 presents the main metabolic pathways for DON in humans. The excretion of DON is fast and occurs within 24 hours, with a large amount excreted in the first 6 hours after ingestion, and DON-15-GlcA was identified as the main DON urinary biomarker (constant ratio of 4/1 within 24 hours, DON-15-GlcA/DON-3-GlcA) (14). For this report and considering that TDI and HBM-GV were established for total DON, all metabolites reported in each study were considered for the determination of the exposure to DON (DON, DOM-1, DON-glucuronides, DON-3G, 3-ADON, 15-ADON).

7.5 Results

7.5.1 Exposure assessment using Human Biomonitoring data

7.5.1.1 Bibliographic data

The excretion of DON as reported by the different studies included in this assessment (mean levels, considering all metabolites reported and the mass balance between the parent compound and the metabolite, expressed as µg/L of total DON) is presented in Figures 2 and 3 with the correspondent distribution by age groups (adults, children, adolescents) and European region (northern, southern, and western). The full list of papers identified in this search can be found in Annex 1.

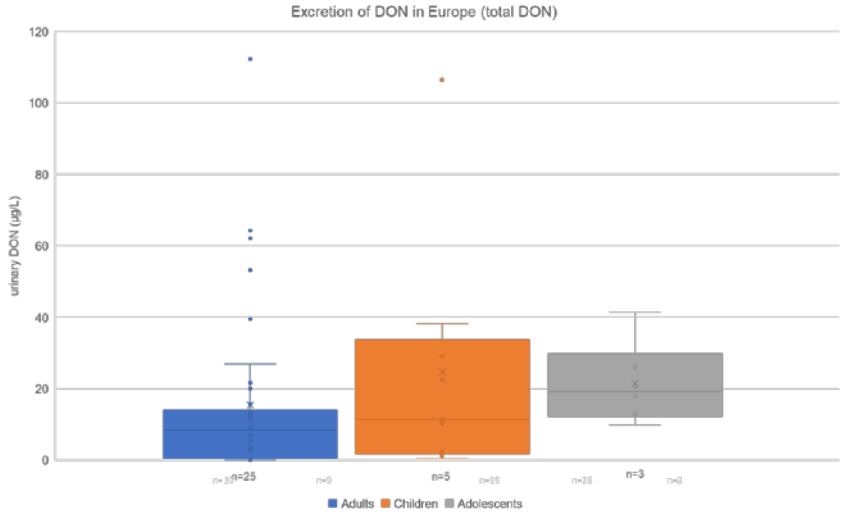


Figure 2: Urinary excretion of DON and metabolites, expressed as total urinary DON levels and by age group, across Europe. n=number of studies

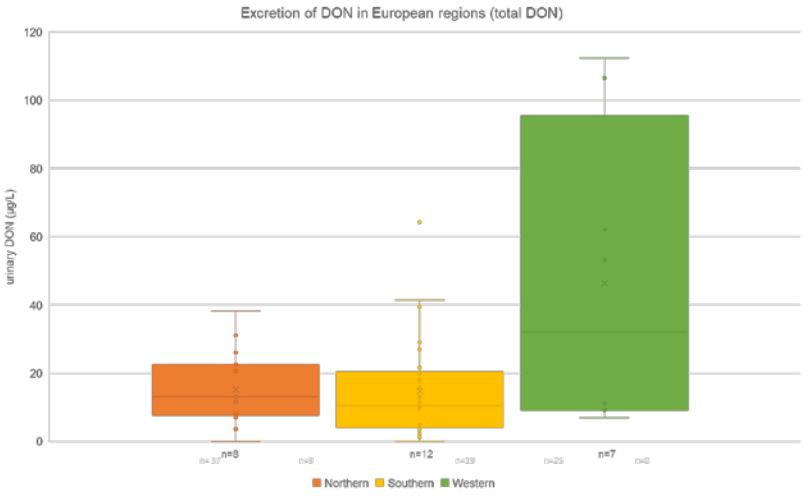


Figure 3: Urinary excretion of DON and metabolites, expressed as total urinary DON levels and by region, across Europe. n=number of studies

In Table 1, data on estimate of exposure to DON of European population is presented. The presented exposure data considered several studies for the general population (n=27) and for workers (n=4). The occupational settings considered in these articles were grain mills, bakeries, and swine production (11,22,23). Additionally, the exposure (µg/kg bw/day) was estimated considering the following variables: age (adults, children, adolescents), specific groups (vegetarians, pregnant women) and the geographical region of Europe (Northern, Southern, and Western Europe).

Table 1: Probable Daily Intake of DON (µg/kg bw/day) calculated for the European population based on bibliographic data.

				DON (µg/kg bw/day)		
Group	**No. of studies**	**No. of participants**	**% of LC data**	**Median**	**Mean**	**Max ****
General population	27	3014	0 – 92 %	0.33	0.72	55.47
Workers*	4	63	0 – 57 %	0.45	0.09	0.63
Age						
Adults	25	2086	0 – 92 %	0.28	0.70	55.47
Children	5	352	0 – 89 %	0.76	1.85	20.62
Adolescents	3	136	0 – 60 %	NR	0.51	3.20
Specific Groups						
Vegetarians	2	124	47 – 78 %	NR	0.67	6.03
Pregnant women	3	124	12 – 60 %	0.69	1.96	55.47
Geographical region						
Northern	8	1279	0 – 78 %	0.07	0.33	8.30
Southern	12	970	4 – 92 %	0.23	0.36	17.42
Western	7	643	0 – 84 %	1.05	2.32	55.47

Results not adjusted for creatinine. Geographical region determined according to the HBM4EU classification. The studies included herewith presented data for the Northern region (United Kingdom and Sweden), Western region (Austria, Belgium, France, Germany) and Southern region (Croatia, Italy, Portugal, Spain). NR = Not reported. LC = Left-censored

* Statistically significant differences were found between exposure of workers (grain mills, bakeries, and swine production) and controls (selected from the general population) to DON.

** Maximum = Result presented as the maximum value obtained within all the studies in each group.

7.5.2 Risk characterisation and uncertainty analysis

7.5.2.1 Bibliographic data

Data on risk characterisation of exposure to DON through hazard quotient calculation (median, mean and maximum) using data reported in the literature and two different approaches - reverse dosimetry (RD) and direct comparison with HBM-GV, for the European population, are presented in the Figure 4.

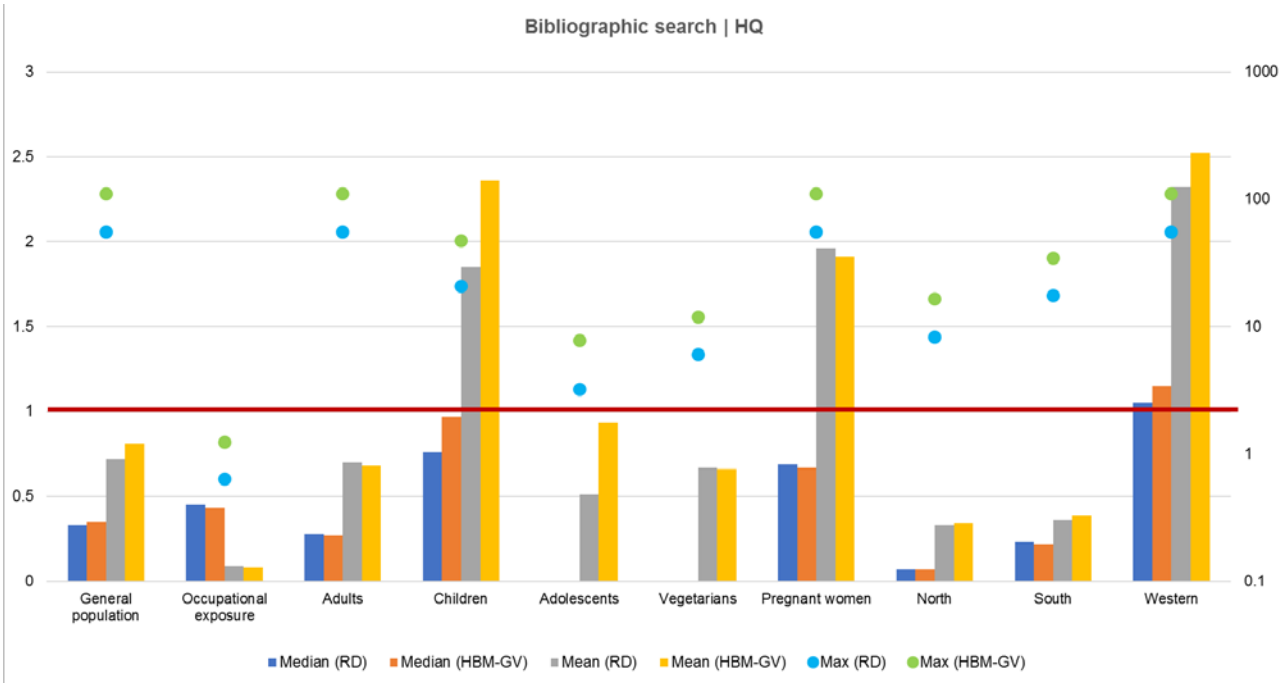


Figure 4: Risk characterisation through hazard quotient calculation. Y-axis = median and mean HQ; Secondary Y-axis = maximum HQ (logarithmic scale); (RD) – using reverse dosimetry value; (HBM-GV) – using Human Biomonitoring guidance value as the reference value

Results presented in Figure 4 show that the exposure to DON for the population of western European region might represent a potential health concern for children, pregnant women, since mean and median levels of exposure corresponded to hazard quotients above one. When considering the general population, where all studies were included except the occupational studies, the median and mean levels of exposure do not represent a potential health concern. However, the individuals presenting the maximum concentrations of total urinary DON are not exempted of risk. The results obtained for the two approaches (reverse dosimetry and direct comparison with HBM-GV) were similar within each other.

7.5.2.2 Sources of uncertainty

Regarding the estimates obtained within this study, it is crucial to clearly state the assumptions considered for the calculations and the associated sources of uncertainty. This information is qualitatively summarised in Table 2.

Table 2: Uncertainty sources identified in the current assessment and the associated impact.

	Source of uncertainty	Impact in the final assessment	
Exposure	Available studies	The studies were designed based on different assumptions, consequently introducing different levels of uncertainties. The studies presented different number of participants. To overcome this issue, the results were weighed for the number of participants in each study, trying to minimise the impact of the size effect. Use of aggregated data (median, mean and maximum levels of DON) reported in each study for the bibliographic data approach of this report.	+/- +/- +
	Left-censored data	Many studies reported high percentage of results below the LODs. For this assessment, the data at individual level was not considered. Therefore, several methodologies for dealing with left-censored data are considered within this assessment.	+
	Uncertainty in estimation of probable daily intake	The sum of all urinary DON biomarkers was considered for estimation of the PDI. The absence of data at individual level led to the use of assumed values for body weight and daily urinary volume.	+/- +
Risk characterisation	Reference dose considered	The use of TDI as reference dose assumes oral exposure as main exposure route. However, as specific groups are considered in the assessment (e.g. workers), other routes could be important (e.g. inhalation). The use of an HBM-GV as a reference value allowed a more accurate estimate of HQ. The use of an HBM-GV as a reference value defined considering 24h urine sampling, for results obtained with first morning urine sampling.	+/- - +/-

+ overestimation; - underestimation; +/- overestimation and/or underestimation.

7.6 Discussion and conclusions

General discussion and conclusions on the work performed

As described both in literature and new aligned studies, the exposure to DON in the European population is generalised, affecting different age and specific groups of the population. According to the available bibliographic data, Eastern region is missing representation in the current assessment. However, according to the predictions already performed regarding the consequences of climate change scenarios, and in addition to South Europe, also Eastern countries could experience high levels of contamination of food raw materials, and consequently, increasing human exposure (24).

Children and pregnant women, which are traditionally considered vulnerable population groups, presented the highest risk. The children group deserves particular attention considering the associated vulnerability and the potential long-term consequences. As already detailed, cereal-based products constitute the main exposure sources to DON (25-28), and significant efforts

should be dedicated to reduce the contamination levels in the foods usually consumed by this age group.

The exposure of workers to DON was also considered in this assessment. Although not representing a potential health risk, it is important to emphasise that the referred studies reported a statistically significant difference between workers and control groups, confirming that the occupational environment can have an important role in increasing the exposure to DON (11,22,23).

The HBM-GV for DON derived under task 5.2 of the HBM4EU project allowed to compare, for the first time, the results obtained under Human Biomonitoring studies for Poland, Iceland, Germany, and Luxembourg. Results from the aligned study conducted in Poland, an eastern country, already showed that the highest percentiles of exposure (P90 and P95) represented a potential health concern since the HQ determined is above one. However, the mean and median levels of exposure were considered as not representing a concern for health. Results obtained for the remaining countries presented a hazard quotient below one for all the percentiles of exposure, thus not representing a potential health concern.

Results in the light of policy questions

PQ: Is the risk associated to human exposure to these mycotoxins characterised?

Results obtained in the present report confirmed that the European population is exposed to DON and that a fraction of this population is, to some extent, exposed to levels that might represent a potential health concern. The risk associated with the exposure to DON for the European population is now characterised.

Regarding the risk characterisation associated with exposure to FB_1, due to a high uncertainty associated with estimates, it was not considered adequate to include it in the present report.

PQ: Is it possible to set a HBM guidance value for mycotoxins?

The establishment of an HBM-GV for DON was performed under the task 5.2, and it is considered of major importance for these assessments, contributing to a significant decrease in uncertainty. The HBM-GV was determined considering a $BMDL_{05}$ of 0.11 mg DON/kg bw/day, reduced body weight gain as the critical effect, and a factor for metabolic conversion of 0.14 (7).

Recommendations for the regulatory risk assessment

The inclusion of mycotoxins' HBM data in risk assessment is important since it represents the internal exposure dose from all sources and by all routes of exposure at individual level, thus reducing the uncertainties associated with risk assessment performed at population level and/ or indirect approaches (e.g., through combination of occurrence in food and food consumption data)(29). The use of HBM data for mycotoxins (and other compounds) implies an extensive knowledge of metabolism and there are still some gaps regarding mycotoxins' toxicokinetic data that may hamper a proper risk assessment. If a risk assessment is developed for regulatory purposes, all these aspects are important for consideration and will assume a high relevance.

The establishment of an HBM-GV is of major relevance for performing a more accurate risk characterisation, allowing a direct comparison of exposure obtained through HBM with a reference value, and reducing the uncertainty in estimates. However, regarding compounds for which a reduced knowledge on metabolism is available, the issue of uncertainty in estimates remains and the limitations of the HBM-GV should be described in detail.

Considering the present report, the use of HBM data for risk assessment encompasses some limitations that are related with the use of data published in several scientific articles, non-

harmonised sample collection and criteria for left-censored data. These limitations were overcome by using HBM data available from the aligned studies, with results obtained in field campaigns using harmonised procedures and analytical methods subjected to a previous quality-control exercise. The limitations may be surpassed in a near future by developing of guidance for setting-up biomonitoring campaigns that allow a proper comparison among studies results and with the HBM-GV.

Future prospects

There are several aspects still requiring further analysis in a near future to allow a more accurate risk assessment:

- The development of more studies to assess exposure in eastern European countries to ensure an adequate exposure assessment throughout all European regions.
- The importance of setting up guidelines for developing biomonitoring studies (for general population and workers) with harmonised procedures to enhance comparison of results between different studies.
- New biomarkers of exposure are not needed since the main metabolites are already identified for DON; however, it is important the development of analytical standards to ensure better analytical measurements and consequently a more accurate exposure assessment.
- The use of 24h urine samples to decrease the uncertainty related to the use of HBM-GV calculated for 24h samples, even considering the associated burden for the participant.

7.7 References

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7.8 Annex 1 – Studies identified in the bibliographic search

By age group | adults

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By age group | children

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By age group | adolescents

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By European region | Northern

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By European region | Southern

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By European region | Western

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By specific group | Pregnant

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By specific group | Vegetarians

Wells, L., Hardie, L., Williams, C., White, K., Liu, Y., De Santis, B., … Sathyapalan, T. (2017). Deoxynivalenol biomarkers in the urine of UK vegetarians. Toxins, 9(7), 1–12. https://doi.org/10.3390/toxins9070196

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8 PAHs full RA report

Risk assessment calculation for Polycyclic Aromatic Hydrocarbons (PAHs): an update using new data from aligned studies

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Acknowledgments

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8.1 Summary

Considering the new HBM data on Polycyclic aromatic hydrocarbons (PAHs) exposure (urinary level of 1-OHPyr) for the general population provided recently by aligned studies under HBM4EU, the aim of the present work was to update the PAHs risk assessment previously performed within D5.5 that was based on published data.

In the present work, the estimation of the of excess life time cancer risk (ELCR) was performed using two approaches. In the first approach, the external exposure was reconstructed, i.e. the Probable Daily Intake (PDI) of Pyrene, from HBM data on 1-hydroxypyrene (1-OHPyr) available in the aligned studies. The PDI of Pyrene intake levels were then translated into PAH4 (BaA, BbF, BaP, CHR) intake levels based on the assumption, that Pyrene intake was a surrogate of PAH4. In the second approach, for comparison, PAH4 intake levels was derived from the country specific food residue and the food consumption data, available at the EFSA reports (2008, 2015). Derived PAH4 intakes were then used as an input to estimate ELCR, using the ECHA-RAC (2018) formula.

The mean intake of PAH4 derived from the EFSA data on the occurrence of PAHs in food was, in general, one order of magnitude higher than that estimated from exposure reconstruction from available HBM4EU data (mean values of 1-OHPyr). This fact was probably due to the conservative nature of the bottom-up approaches, based on the EFSA data, compared to the one that was based on HBM data. Consequently, the ELCR estimates based on EFSA's PAH4 intake levels were higher than those calculated from intake levels based on HBM data exposure reconstruction. Thus, that EFSA's bottom-up approach may be considered as the worst-case scenario for exposure estimation. The ELCR mean values estimated from HBM data ranged from $3.9x10^{-6}$ to $3.2x10^{-5}$. Considering the indicative tolerable risk level of 10^{-6} for the general population proposed by the European Commission (EC 2001), the ELCR results obtained in the present work were of concern for 4 of the 7 countries included in aligned studies (Luxembourg, France, Czech Rep and Poland), all with ELCR estimation in the 10^{-5} range.

However, it should be noted that for this estimation, the approach used took into account only the oral route of exposure, i.e. it assumed that most of the intake occurs by oral route. Since it is well known that inhalation also contributes for PAHs exposure at a similar proportion (roughly 50%), the ELCR values calculated are likely to underestimate the real cancer risk. Thus, for a more realistic estimate, the inhalation route of exposure should be addressed properly in future risk assessment studies. Further, an assumption that the pyrene intake derived from the urinary 1-OHPyr is representative of PAH4 intake should be taken with caution, as well as all uncertainties that were not addresses in this work. As new aligned studies provided also other PAHs metabolites, such as 1-hydroxynaphthalene, 9-hydroxyfluorene 4-hydroxyphenanthrene, these might be used in future RA studies to improve uncertainties and eliminate potential doubts as much as possible. To allow the RA based on those metabolites it is also of the greatest importance to deliver the health-based Guidance Values (HBM HBGVs) for PAHs metabolites that would help to interpret the HBM results in much more reliable health risk context.

It is concluded that the most recent values obtained in the aligned studies under the HBM4EU Initiative showed that the levels of internal exposure of the European adult population were similar to those from previous studies published in the literature. There were no indications that the newly obtained HBM data for 1-OHPyr were substantially lower than the ones previously measured and reported in the open literature. In addition, while comparing the risk estimations with the ones formerly estimated and presented in D5.5, the risk levels now estimated are at the same order of magnitude, with an exception of smokers (10^{-4}), considering solely oral (dietary) exposure. From

the health policies questions perspective, further efforts should be envisaged for reducing intake and potential contamination with PAHs from various sources.

8.2 Introduction

8.2.1 Background information

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants generated primarily during the incomplete combustion of organic materials (e.g. coal, oil, petrol, and wood). Emissions from anthropogenic activities predominate (automobile emissions and cigarette smoke); nevertheless, some PAHs in the environment originate from natural sources (e.g. open burning, natural losses or seepage of petroleum or coal deposits, and volcanic activities). Humans can be exposed to PAHs through different routes. For the general population, the major routes of exposure are from food and inhaled air, while in smokers, the contributions from smoking and food may be of a similar magnitude. Food can be contaminated by environmental PAHs that are present in air, soil or water, by industrial food processing methods (e.g. heating, drying and smoking processes) and by home food preparation (e.g. grilling and roasting processes) (Tschersich et al., 2018). Occupational exposure to PAHs may occur by inhalation and also by dermal route that, in specific workplaces, can have an important role in the total occupational uptake of PAHs (SCOEL, 2016).

In the previous work, performed and published under deliverable D5.5 in 2019 (Santonen et al., 2019), the estimation of excess life time cancer risk (ELCR) for Polycyclic Aromatic Hydrocarbons (PAHs) was performed for inhalation and oral exposure to PAHs in general population and for inhalation exposure in occupational settings. In these estimates, the ECHA-RAC (RAC-Committee for Risk Assessment (2018) dose-response relationship, Table 1, was used. In inhalation exposure of general population, ELCR for lung cancer was estimated, based on airborne benzo[a]pyrene (BaP) levels and in occupational inhalation exposure, urinary levels of 1-OHPyr in workers was back-calculated to corresponding airborne BaP levels. In dietary (oral) exposure of general population, ELCR for cancer[15] was on the benchmark dose lower confidence limit (BMDL10) for exposure to PAH416 and PAH817 (EFSA, 2008), Table 1. In this estimation, an assumption was made, that pyrene was an indirect marker of exposure to PAH4 or PAH8 mixtures and that for this purpose, the median daily intake could be used, as back-calculated from urinary 1-OHPyr for some EU countries (and EU average) within WP12.5 (Sarigiannis & Karakitsios, 2018). However, these former estimates were only approximate and informative (for detailed information see Deliverable D5.5; Santonen et al., 2019; Annex E).

The general goal of the present document is to update the previous work, briefly described above. In this update, ELCR for oral (dietary) exposure of general population was estimated, using the same ECHA-RAC dose-response relationship, shown in Table 1. In the present work, the following approach was used:

- Estimate the external exposure (Probable Daily Intake, PDI) to pyrene, based on HBM data on 1-OHPyr from aligned studies, using reverse dosimetry.
- Estimate ELCR for PAH4 intake, based on association of pyrene intake with PAH4, using ECHA-RAC dose-response relationship for oral exposure of general population and the $BMDL_{10}$ for PAH4.

[15] Tumours of the liver, lung, forestomach, small intestine, hemangiosarcomas, histiocytic sarcomas and sarcomas of the mesentery, forestomach, skin and kidney (EFSA, 2008)
[16] PAH4: BaA, BbF, BaP and CHR (EFSA, 2008)
[17] PAH8: PAH4 + BkF, BghiP, DBahA and IP (EFSA, 2008)

Dietary exposure to pyrene was back-calculated from urinary 1-OHPyr levels for the general population, provided recently by aligned studies under HBM4EU. Further, in this approach, the pyrene intake levels back calculated from the available 1-OH-pyr HBM data were translated into PAH4 intake levels (using the methodology described below) to be used as an input to the ECHA-RAC formula. In addition, food residue data for PAH4 was gathered, based on EFSA's L1-Level for some countries across Europe (EFSA 2015), as well as on food consumption data of the respective countries. The relative proportion of Pyr to PAH4 was used to deliver the country specific PAH4 estimates.

As a reference, the World Health Organisation (WHO) guidance was considered, suggesting that the unit risk of lung cancer is 87 x 10^{-6} per ng BaP/m^3 for lifetime exposure (WHO, 2000). In addition, under most regulatory programs, an ELCR of 10^{-6} or less was considered as virtually safe, while ELCR greater than 10^{-4} was considered as high risk (Ambient air pollution by PAHS (European Commission, 2001). Further, the indicative tolerable risk level for the general population was proposed by the EC (2016) at levels of 10^{-6} (http://ec.europa.eu/growth/content/workshop-acceptable-level-risk-workers-and-consumers-exposed-carcinogenic-substances-0_en).

The policy questions enunciated in the Scoping document for 2018, Deliverable Report D4.2 June 2017, page 117 (Tschersich et al., 2018) are as follows:

- How high is the current (year 2012 or more recent) exposure (both external and internal) of the EU population (working and general) to data-rich substances?
- Are the overall exposure levels in the general population, children, and pregnant women above any health-relevant assessment levels (reference dose or HBM Guidance Values)?
- What are knowledge gaps and related research needs for data-rich substances to answer the questions above satisfactorily in the following years (Year 3)? Can the identified knowledge gaps be mended based on existing data or by extension of current good HBM?

8.3 Methodology

A practical way to estimate the risk associated with PAHs, was (a) to estimate intake levels of the main carcinogenic PAHs based on urinary 1-OHPyr HBM levels and (b) to translate this intake into ELCR for the PAH4. The following steps were performed.

First, the intake of pyrene was reconstructed based on urinary HBM data in which 1-OHPyr was measured. For exposure reconstruction, HBM data were retrieved from both the HBM4EU repository data, as well as the ones from the aligned studies, including studies on both the general population and the occupationally exposed individuals. 1-OHPyr is a pyrene metabolite and is therefore an indirect marker of exposure to PAH mixtures that include BaP. As described by the ECHA RAC-Committee for Risk Assessment in 2018, a good correlation between 1-OHPyr in urine and BaP or total PAHs intake is confirmed (RAC, 2018; adopted from Unwin et el., 2006). This has been also verified on the modelling work that has been carried out in HBM4EU and is presented in the deliverable "AD12.3 - Exposure model testing results", it was found that, as a result of multimedia transfer of these compounds, they are also well correlated in food items.

The second step was to perform a literature review, with aim to transform pyrene intake into PAH4 intake. For this purpose, food residue data for Pyr and the PAH4 (BaP, BbF, BaA and CHR) was gathered based on EFSA's L1-Level for many countries across Europe (EFSA 2008), as well as the country food consumption data of the respective countries (Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Netherlands, Romania, Spain, Sweden and UK) (EFSA 2015). Daily intake was calculated based on these data and a relation between daily (dietary) intake of pyrene and PAH4 was drawn,

accounting also for the differences among the various countries. L1-level is the first level hierarchy of food classification proposed by EFSA comprising the major food families (EFSA, 2015). Based on the above, the pyrene intake of the available HBM data from the HBM4EU database was transformed into oral PAH4 intake.

8.3.1 Exposure reconstruction based on HBM data

The intake estimates were based on the exposure reconstruction using the available HBM data on urinary 1-OHPyr. The method was performed according to the following steps:

- The process starts from external exposure related data (such as food residues, concentration in ambient and indoor air, dietary schedule, inhalation rate etc.) which are fed into the external exposure model, accounting for the various potential pathways and routes of exposure. In the specific cases, exposure is dominated by diet.
- This in turn provides input to the PBTK model, taking into account the duration and the magnitude of exposure from all the exposure routes (inhalation, skin and oral route);
- The result of the PBTK model simulation (taking also into account the distribution of PBTK parameters, e.g. inter-individual variability in clearance), is then evaluated against the Human Biomonitoring data distributions. Based on the outcome of the comparison, the optimisation algorithm changes the exposure model input parameters following each iteration, so as to achieve convergence to the biomonitoring data;
- More detailed information on exposure parameters reduces uncertainty in back-calculating doses from biomarker information, resulting in faster and more efficient convergence;
- Several iterations are repeated, until the error between the predicted and the actual biomonitored data is minimised. The error minimisation procedure uses genetic or differential evolution Markov chain algorithms to ensure that the exposure profile to which the overall schema converges is mathematically the optimal profile that explains the variance in the Human Biomonitoring measurements.

8.3.2 ELCR calculations

The ECHA-RAC dose-response relationships for the carcinogenic properties of PAHs mixture as BaP, PAH4 and PAH8 (ECHA-RAC, 2018) and various exposure routes and population are shown in Table 1.

Table 1: Overview of reference dose-response relationships for the carcinogenic properties of PAHs mixture CTPHT (as BaP, PAH4 and PAH8) (ECHA-RAC, 2018)

Route	Cancer type	Lifetime excess risk	
		Workers	General population
Inhalation	lung cancer	5.6×10^{-6} per ng/m^3 [(a)]	3.0×10^{-5} per ng/m^3
	bladder cancer	4×10^{-6} per ng/m^3 [(a)]	2.1×10^{-5} per ng/m^3
Dermal	skin cancer	1.3×10^{-3} per ng BaP/cm^2/day	Not derived [(c)]
Oral	Cancer[1]	Not relevant	2.06×10^{-3} per µg PAH4/kg bw/day 1.43×10^{-3} per µg PAH8/kg bw/day

[a] Exposure levels in air can also be derived from urinary 1-OH-Pyr or 3-OHBaP biomonitoring data using the relationships:

- urinary post-shift concentration of 3-OHBaP (µmol/mol creatinine) = 0.001835 × 8h TWA BaP concentration in air (µg/m^3) + 0.1729
- urinary post-shift concentration of 1-OHPyr (µmol/mol creatinine) = 11.1 × 8h TWA BaP concentration in air (µg/m^3) + 1.13

[c] No significant exposure of the general population by the dermal route is envisaged. Therefore, no dose-response was derived.

In oral exposure of general population, ELCR estimate is based on the benchmark dose lower confidence limit ($BMDL_{10}$) derived by the EFSA (2008). $BMDL_{10}$ is related to 10% response (0.1) for tumours of the liver, lung, forestomach, small intestine, hemangiosarcomas, histiocytic sarcomas and sarcomas of the mesentery, forestomach, skin and kidney from the 2-year oral carcinogenicity study on coal tar mixtures (CTPHT) performed by Culp et al. (1998; referred in the EFSA, 2008). $BMDL_{10}$ for PAH4 amounted to 340 µg/kg b.w./day and in combination with an allometric scaling factor of 7, the unit of ELCR has been estimated (RAC-Committee for Risk Assessment, 2018b) as follows (and shown in Table 1):

$$\text{PAH4: ELCR} = 0.1 \times 7/340 = 2.06 \times 10^{-3} \times \text{exposure dose per µg/kg b.w./day,}$$

and where ‘exposure dose’ in this case refers to the median dietary (oral) intake of PAH4 that was back-calculated as described above.

8.4 Hazard assessment

This part was described in D5.5. Here, a brief overview is provided. In the past decade, PAHs were evaluated by the International Programme on Chemical Safety (IPCS) (WHO/IPCS, 1998), the Scientific Committee on Food (SCF) (EC, 2002) and by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (FAO/WHO, 2005). Lung, bladder and skin cancers are identified as the key cancer risk endpoints for exposure to PAHs (International Agency for Research on Cancer, 2010). PAH mixtures and BaP are genotoxic carcinogens, for which safe health-based exposure limits cannot be derived for the general population. Several epidemiologic studies have shown increased cancer mortality in workers exposed to PAH mixtures that have been already referred in the Scoping document for 2018; Deliverable Report D4.2 June 2017 pages 101-103 (Scoping document, 2017). More recently, in 2018, Risk Assessment Committed (RAC) from European Chemicals Agency (ECHA) elaborated an overview of reference dose-response relationships for the carcinogenic properties of CTPHT (coal tar, pitch, high temperature) based on BaP concentration (RAC, 2018).

8.5 Exposure assessment using Human Biomonitoring data

In Table 2, HBM data on urinary 1-OHPyr in µg/L from aligned studies are shown. For potential comparison, data is also shown in units, adjusted for creatinine (crt). While comparing these data with urinary 1-OHPyr values, reported in our former work in D5.5 (Table 4), no significant difference was observed, i.e., a pooled mean of 0.097±0.044 µg/g crt compared to 0.113±0.078 µg/g crt in the aligned studies (p= 0. 693, Student’s t-test). Additionally, the percentiles distribution of data is presented as supplementary material.

Table 2: HBM data on 1-OHPyr (geometric mean) from aligned studies, used in exposure reconstruction calculations.

Country	Study	Type of sampling	Geometric Mean (µg/g crt)	Geometric Mean (µg/L)	N	Current smoker N	Gender (Male/Female) N
France	A_ANSP_ESTEBAN	First Morning	0.1303	0.1193	393	114	177/216
Poland	A_NIOM_POLAES	Spot	0.2686	0.3008	228	30	70/158
Iceland	A_UI_DIET_HBM	Spot	0.0327	0.0375	198	14	87/111
Czech Republic	A_MU_(C)ELSPAC	First Morning	0.0703	0.0888	300	36	145/155
Croatia	A_CIPH_HBM in Croatia	First Morning	0.0492	0.0700	300	91	141/159
Switzerland	A_SWISS TPH_HBM4EU-study	First Morning	0.0783	0.0742	300	66	162/138
Germany	A_UBA_ESB	24h	0.0653	0.0451	984	89	488/496
Luxembourg	A_LNS_Oriscav-Lux2	Spot	0.2067	0.3572	210	36	99/111

Only data from adults, unstratified. Biomarker data quality assured by HBM4EU QA/QC program. Crt- creatinine.

8.6 Risk characterisation and uncertainty analysis

Estimated ELCR, based on HBM data on 1-OHPyr and reverse dosimetry, are presented in Table 3.

Table 3: ELCR values as determined based on new data from HBM4EU aligned studies in comparison with bottom-up approach using the mean food consumption and the PAHs residues in food items, based on EFSA data (EFSA, 2008)

Country	Study	Data	PAH4 intake using HBM data µg/kg_bw/day	PAH4 intake (EFSA, 2008, 2015) µg/kg_bw/day	ELCR*
France	A_ANSP_ESTEBAN	median	0.0027		5.56×10^{-06}
		mean	0.0049	0.022	1.01×10^{-05}
		max	0.0208		4.29×10^{-05}
Poland	A_NIOM_POLAES	median	0.0098		2.01×10^{-05}
		mean	0.0142	0.028**	2.93×10^{-05}
		max	0.0527		1.09×10^{-04}
Czech R.	A_MU_(C)ELSPAC	median	0.0025		5.08×10^{-06}
		mean	0.0055	0.027	1.12×10^{-05}
		max	0.0135		2.79×10^{-05}
Croatia	A_CIPH_HBM in Croatia	median	0.0019		3.91×10^{-06}
		mean	0.0028	0.028**	5.71×10^{-06}
		max	0.0099		2.05×10^{-05}
Switzerland	A_SWISSTPH_HBM4EU-study Switzerland	median	0.0025		5.13×10^{-06}
		mean	0.0039	0.028**	8.08×10^{-06}
		max	0.0119		2.45×10^{-05}
Germany	A_UBA_ESB	median	0.0014		2.83×10^{-06}
		mean	0.0019	0.028	3.92×10^{-06}
		max	0.0063		1.30×10^{-05}
Luxembourg	A_LNS_Oriscav-Lux2	median	0.0129		2.65×10^{-05}
		mean	0.0156	0.028**	3.21×10^{-05}
		max	0.0508		1.05×10^{-04}

*based on PAH4 dose-response: 2.06×10^{-03} (RAC, 2018).
**For countries that data from EFSA where not available, Germany has been used a proxy

The ELCR mean values showed in Table 3 ranged from 3.9×10^{-6} in Germany to 3.2×10^{-5} in Luxembourg. It could be assumed that the mean uptake estimated by EFSA based on the occurrence of PAHs in food was, in general, one order of magnitude higher than that estimated from the available HBM4EU data. Thus, this bottom-up approach may be considered as the worst-case scenario in exposure estimation.

8.7 Conclusions

The most recent values obtained in the aligned studies by HBM4EU, show that the levels of internal exposure in the European population, in adults, are similar to those from previous studies published in the literature. In addition, while comparing these estimates with the former estimates in D5.5, it could be concluded that in general the risk levels were estimated at the same order of magnitude, with an exception for smokers (10^{-4}), considering oral (dietary) exposure. We have no indications that the newly obtained HBM data for 1-OHPyr were substantially lower than the ones previously measured and reported in the open literature.

Considering the indicative tolerable risk level for the general population proposed by the EC (2016) at levels of 10^{-6} the ELCR results obtained in the present work for 4 of the 7 countries included in aligned studies (Luxembourg, France, Czech Rep and Poland), all in the 10^{-5} range, are of concern.

The mean uptake estimated by EFSA based on the occurrence of PAHs in food is, in general, one order of magnitude higher than that estimated from exposure reconstruction based on the available HBM4EU data (mean values). This is probably due to the conservative nature of the bottom-up approaches to exposure estimation, compared to the one that is based on HBM data reconstruction. As such, the ELCR estimates based on EFSA's PAH4 intake levels is higher than the risk calculated from intake estimates based on HBM data exposure reconstruction, because the intake estimates are higher.

Further, an assumption that pyrene intake is represented by the biomarker 1-OHP in urine and presented as PAH4 intake needs to be taken with caution, since there are some doubts and uncertainties whether 1-OH pyrene is a reliable bioindicator of measured dietary polycyclic aromatic hydrocarbon under normal conditions (Viau et al., 2002). In addition, pyrene is not classified as carcinogenic and monitoring of its metabolite for the purpose of risk assessment is only based on the assumption that the level of 1-OHPyr correlate to some extent with BaP and other carcinogenic species co-occurring commonly with pyrene in PAHs mixtures. In addition, exposure to PAHs mixture may modify metabolism or induce different effects, therefore a single metabolite may not adequately characterise exposure to PAHs.

New aligned studies provide recent data on other 11 PAHs and metabolites, such as 1-hydroxynaphthalene, 9-hydroxyfluorene 4-hydroxyphenanthrene. However, there are no available HBM health-based Guidance Values (HBM HBGVs) for none of PAHs metabolites that would help to interpret the HBM results and to put the results in a health risk context. Deriving future HBGVs for these metabolites would allow further RA for these compounds.

In this estimation, an approach was used, taking into account only the oral route of exposure, i. e. the fact that most of the intake was assumed by oral route. However, inhalation contributes a similar proportion (roughly 50%), so inhalation is probably important factor and should be discussed properly in future risk assessment studies.

In general, the majority of PAHs exposure is assumed to be via diet, but in smokers, inhalation contributes a similar proportion (roughly 50%), so inhalation is probably an important factor and should be discussed properly. Potential measures to reduce intake of PAHs could include avoiding contact of foods with flames, and cooking with the heat source above rather than below the food. Efforts should be made to reduce contamination with PAHs during drying and smoking processes.

8.7.1 Conclusions in the contexts of the policy questions (PQs)

PQ1: How high is the current (year 2012 or more recent) exposure (both external and internal) of the EU population (working and general) to data-rich substances?

The most recent values obtained in the HBM4EU aligned studies show that the levels of internal exposure in the European population, in adults of the general population, are similar to those from previous studies published in the literature. We have no indications that the newly obtained HBM data for 1-OHPyr were substantially lower than the ones previously measured and reported in the open literature.

PQ2: Are the overall exposure levels in the general population, children, and pregnant women above any health-relevant assessment levels (reference dose or HBM Guidance Values)?

No analysis was done on stratified data since there are no reference values for that.

Considering the indicative tolerable cancer risk level for the general population proposed by the EC (2001) at levels of 10-6, the ELCR results obtained in the present work for 4 of the 7 countries included in the aligned studies (Luxembourg (A_LNS_Oriscav-Lux2), France (A_ANSP_ESTEBAN),Czech Republic (A_MU_(C)ELSPAC) and Poland(A_NIOM_POLAES)), all in the 10-5 range, are of concern.

The mean dietary intake estimated by EFSA based on the occurrence of PAHs in food is, in general, one order of magnitude higher than that estimated daily intake from exposure reconstruction based on the available HBM4EU data (mean values). This is probably due to the conservative nature of the bottom-up approaches to exposure estimation, compared to the one that is based on HBM data reconstruction. As such, the ELCR estimates based on EFSA's PAH4 intake levels is higher than the risk calculated from intake estimates based on HBM data exposure reconstruction, because the dietary intake estimates are higher.

Further, an assumption that pyrene intake is represented by the biomarker 1-OHP in urine and presented as PAH4 intake needs to be taken with caution, since there are some doubts and uncertainties whether 1-OH pyrene is a reliable bioindicator of measured dietary polycyclic aromatic hydrocarbon under normal conditions (Viau et al., 2002). In addition, pyrene is not classified as carcinogenic and monitoring of its metabolite for the purpose of risk assessment is only based on the assumption that the level of 1-OHPyr correlate to some extent with BaP and other carcinogenic species co-occurring commonly with pyrene in PAHs mixtures. In addition, exposure to PAHs mixture may modify metabolism or induce different effects, therefore a single metabolite may not adequately characterise exposure to PAHs.

PQ3: What are knowledge gaps and related research needs for data-rich substances to answer the questions above satisfactorily in the following years (Year 3)? Can the identified knowledge gaps be mended based on existing data or by extension of current good HBM?

In this estimation, an approach was used, taking into account only the oral route of exposure, i. e. the fact that most of the intake was assumed by oral route. However, inhalation contributes a similar proportion (roughly 50%), so inhalation is probably important factor and should be discussed properly in future risk assessment studies.

New HBM4EU aligned studies provided recent data on other 11 PAHs and metabolites, such as 1-hydroxynaphthalene, 9-hydroxyfluorene 4-hydroxyphenanthrene. However, there are no available health-based guidance values for internal exposure for PAHs metabolites that would help to interpret the HBM results, and to put the results in a health risk context. Deriving future HBGVs for these metabolites would allow further RA for these compounds.

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8.9 Supplementary data from aligned studies

HBM data on 1-OHPyr from aligned studies, in urine, in percentiles.

Study	Type of sampling	Urine 1-OHPyr concentration (µg/L)						
		P05	P10	P25	P50	P75	P90	P95
A_ANSP_ESTEBAN	First Morning	0.043	0.055	0.0742	0.1178	0.2111	0.3571	0.556
A_NIOM_POLAES	Spot	0.09	0.111	0.1684	0.2408	0.4323	0.7027	1.0234
A_UI_DIET_HBM	Spot	-2	-2	0.0207	0.0316	0.0484	0.0704	0.0962
A_MU_(C)ELSPAC	First Morning	0.02	0.032	0.0475	0.0693	0.1037	0.1606	0.2322
A_CIPH_HBM in Croatia	First Morning	-1	0.017	0.031	0.0523	0.0914	0.1485	0.2057
A_SWISS TPH_HBM4EU-study	First Morning	0.023	0.032	0.048	0.0769	0.1271	0.2226	0.2842
A_UBA_ESB	24h	0.021	0.029	0.041	0.0611	0.098	0.1759	0.2722
A_LNS_Oriscav-Lux2	Spot	0.066	0.092	0.1444	0.2053	0.3567	0.4767	0.5941

Study	Type of sampling	Urine 1-OHPyr concentration (µg /g crt)						
		P05	P10	P25	P50	P75	P90	P95
A_ANSP_ESTEBAN	First Morning	0.031	0.04	0.066	0.108	0.222	0.419	0.597
A_NIOM_POLAES	Spot	0.085	0.101	0.1665	0.302	0.5312	0.8718	1.3565
A_UI_DIET_HBM	Spot	-2	-2	0.02	0.04	0.07	0.123	0.15
A_MU_(C)ELSPAC	First Morning	0.02	0.039	0.06	0.09	0.16	0.241	0.3105
A_CIPH_HBM in Croatia	First Morning	-1	0.02	0.04	0.07	0.14	0.28	0.36
A_SWISS TPH_HBM4EU-study	First Morning	0.02	0.03	0.04	0.07	0.12	0.201	0.2715
A_UBA_ESB	24h	0.02	0.02	0.03	0.04	0.07	0.11	0.17
A_LNS_Oriscav-Lux2	Spot	0.075	0.117	0.212	0.415	0.65	0.96	1.2303

9 UV-filter BP-3 full RA report

Human Biomonitoring in risk assessment of Benzophenone-3: update with aligned study data

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Acknowledgements

Sanna Lignell, Swedish Food Agency

Susana Pedraza (ISCIII)

Amrit Kaur Sakhi, Line Smastuen, Cathrine Thomsen, Norwegian Institute of Public Health

Phillipp Schmidt, Nina Vogel, German Environment Agency

and all HBM4EU aligned study data owners for providing the data that were used in this assessment.

9.1 Summary

Benzophenone-3 is a UV-filter that is widely applied in a variety of consumer products, principally sunscreens and other cosmetic products, and in lower amounts in paints and coatings. Due to concerns on the endocrine disruptive properties of benzophenone-3 (BP-3), there is a consumer concern for a possible risk through the use of BP-3 containing products.

Under the scope of the HBM4EU project, a risk assessment was performed of BP-3 based on Human Biomonitoring data (Annex). In the previous HBM4EU deliverable, the biomonitoring data from three studies literature studies performed in the period 2010-2013 was used. This risk assessment found an increased risk for the highly exposed population, but not for typical exposure. After this study, new Human Biomonitoring (HBM) measurements were performed within the HBM4EU project including data on BP-3, with a sampling period ranging from 2014-2018. This report provides an update of the risk assessment with this new data. In addition, the point of departure (POD) was updated in line with the 2021 Scientific Committee for Consumer Safety (SCCS) opinion. This resulted in the use of a lower POD, but also a lower assessment factor due to the difference in endpoint. As the difference in the resulting provisional HBM guidance value was relatively small (less than 3-fold decrease), the outcome of the risk assessment on the studies from literature stayed essentially the same.

Six of the aligned studies conducted from 2014 to 2018 within HBM4EU performed new measurements of benzophenones, including BP-3. The question was whether there would be any change in the exposure, in particular due to the lower maximum allowed concentration of BP-3 in sunscreen products since 2017. The outcome of the new risk assessment showed that indeed the P50 and P95 are lower in the more recent measurements. Of the six new studies the highest Risk Characterisation Ratio (RCR) at the P95 was 0.2, where it was 1.15 in the previous assessment. However, an important note is that the highest exposed cohort in the previous assessment was not included in the aligned studies, while the other cohorts from literature had similar exposure levels to those in the aligned studies. In general, higher exposure levels were found in woman than in men in the new studies. No clear influence was found of age or sample regime.

This preliminary risk assessment of BP-3 implied that in general there is no risk indicated to the population included in the aligned studies. It also shows that there are notable differences in exposure between groups, with the most highly exposed groups approaching (but not surpassing) the acceptable risk levels for BP-3. This analysis was greatly aided by the coordination of HBM studies within HBM4EU, which ensured that measurements were performed and analysed in a standardised way that enabled a meaningful comparison. This study notes the value of empirical HBM data and highlights the importance of the HBM4EU initiative in advancing and coordinating biomonitoring research in Europe.

9.2 Answers to policy questions

The aim was to assess the exposure levels in the European population and to determine whether those exposure levels are safe in relation to the toxicological properties of benzophenone-3. Additionally, this report considered how Human Biomonitoring data can inform the risk assessment case of benzophenone-3, whether the necessary tools can be acquired, and if this information is useful for policymakers. By answering these questions, we respond to policy questions 2, 6, and 9 from the scoping document on UV-filters:

What are current levels of exposure of the EU population to benzophenone UV-filters?

How effective was the restriction of BP-3 in reducing exposures in the EU population?

How can HBM4EU results feed into regulatory decisions and risk assessments (ECHA and EFSA)?

Exposure was assessed with a meta-analysis of the biomonitoring data and in six of the aligned studies. The 3 studies from literature included BP-3 concentrations measured in urine samples in cohorts from Denmark, Belgium, and Spain that were sampled between 2010 and 2013. The six aligned studies that included BP-3 were performed in Germany, Luxembourgh, Sweden, Norway, Spain, and Poland and samples were taken between 2014 and 2018.

The meta-analysis resulted in exposure estimates ranging from 0.60 to 4.40 µg/g creatinine for the average population, and 16.30 to 392.00 µg/g creatinine for the highly exposed population. In the aligned studies, the typical case BP-3 exposure based on the P50 ranged from <LOQ to 3.68 µg/g creatinine and the reasonable worst-case exposure, based on the P95, ranged from 18.97 to 68.83 µg/g creatinine. The most sensitive endpoint for the hazardous properties of benzophenone-3 was reproductive toxicity. The derived provisional HBM guidance value was 340 µg/g creatinine. The outcome of the new risk assessment showed that indeed the P50 and P95 are lower in the more recent measurements. Of the six new studies the highest RCR at the P95 was 0.2, where it was 1.15 in the previous assessment. However, an important note is that the highly exposed cohort in the previous assessment was not included in the aligned studies, while the other cohorts from literature had similar exposure levels to those in the aligned studies. In general, higher exposure levels were found in woman than in men in the new studies. No clear influence was found of age or sample regime.

This preliminary risk assessment of BP-3 implied that in general there is no risk indicated to the population included in the aligned studies. It also shows that there are notable differences in exposure between groups, with the most highly exposed groups approaching (but not surpassing) the acceptable risk levels for BP-3. This information is valuable input for regulatory authorities as it provides insight in real-life exposures, which can be used to refine regulatory risk assessments and assess the effectiveness of regulation.

9.3 General population risk assessment of UV-filter benzophenone-3

9.3.1 Introduction

The HBM4EU project has selected chemicals for research activities under the project in prioritisation rounds based on health and regulatory interest. In the second prioritisation round, the benzophenone-type UV filters were selected (HBM4EU, 2019). Benzophenones are lipophilic phenols chemically synthesised for their UVA and UVB absorbing and dissipating properties. Twelve designated benzophenone derivatives (benzophenone 1-12) are commercially available.

Toxicity of most benzophenone derivatives is not well investigated, but benzophenone-3 (2-hydroxy-4-methoxy-benzophenone; BP-3) is extensively studied for regulatory purposes as well as out of scientific interest. Of the commercially available benzophenones, BP-3 is the most widely applied UV-filter in consumer products (DEPA, 2015; Liao & Kannan, 2014). The current European cosmetics regulation permits maximum concentrations of 6% BP-3 as an active ingredient in sunscreens and up to 0.5% in other cosmetic products to protect formulations (EC, 2020). These maximum concentrations are effective since 2017. BP-3 can also be added to paints and coatings as a UV-stabiliser, usually in concentrations between 0.1 and 1.0% (DEPA, 2015). Under the scope of the HBM4EU project, the current study focuses on BP-3, based on usage and data availability.

Because of the widespread use of BP-3 in a variety of products, human aggregated exposure likely occurs, including exposure combined and accumulated from all sources and through different exposure routes (Calafat et al., 2008; DEPA, 2015). For such aggregated exposure, HBM can be especially relevant for performing a risk assessment. A large body of evidence on human data implies rapid absorption and systemic exposure after oral and dermal administration of BP-3 (Gonzalez et al., 2006; Hayden et al., 1997; Janjua et al., 2008; Janjua et al., 2004). After absorption, BP-3 is partially metabolised. Suggested metabolites of BP-3 are BP-1, BP-2, BP-8, and 4-OH-BP, with BP-1 strongly indicated as the main metabolite of BP-3 (Wang & Kannan, 2013). In both animals and humans, BP-3 and its metabolites are primarily eliminated through urinary excretion in free and conjugated forms (Hayden et al., 1997; Janjua et al., 2004; Okereke et al., 1993; Sarveiya et al., 2004). Total deconjugated BP-3 concentration is commonly measured in urine as a non-invasive biomarker for internal exposure.

Information on systemic absorption of BP-3 from consumer products raised concern that there might be a risk for consumers (EWG, 2019). BP-3 is registered under the EU REACH Regulation (Registration, Evaluation, Authorisation, and Restriction of Chemicals) (EC No. 1907/2006). REACH requires companies to register their substances with information on hazardous properties and chemical safety reports. However, the US Food and Drug Administration and the Danish Environmental Protection Agency concluded that for many sunscreen ingredients, including BP-3, not enough safety information is available (DEPA, 2015; Matta et al., 2019), especially for carcinogenicity and endocrine disruption (CoRap, 2014; EWG, 2019). Because of the latter, BP-3 is currently under substance evaluation for endocrine-disruptive (ED) properties (ECHA, 2020). In its recent reevaluation, the Scientific Committee on Consumer Safety (SCCS) concluded that the available evidence on the ED properties of BP-3 is still inconclusive, or at most equivocal (SCCS, 2021).

The risk assessments of BP-3 in the Cosmetics legislative framework have always been performed on calculated exposure, which uses the concentrations in cosmetic products and dermal absorption to estimate the Systemic Exposure Dose. Incorporation of biomonitoring information in chemical risk assessment is not common practice yet, and its value in risk assessment is largely unexplored. In the previous HBM4EU deliverable, a risk assessment of BP-3 was performed on biomonitoring data from three studies performed in the period 2010-2013. This risk assessment found an increased risk for a highly exposed population, but not for the typical exposure. However, it is noteworthy that in 2017 the maximum allowed concentration of BP-3 in sunscreens was lowered from 10% to 6% in the EU. Thus it is likely that BP-3 exposure from this source has been reduced. Within HBM4EU, new HBM measurements were performed including data on BP-3, with a sampling period ranging from 2014-2018. This report provides an update of the risk assessment with this new data. In addition, the point of departure (POD) was updated in line with the 2021 SCCS opinion.

9.3.2 Methods

Within this report two risk assessments are performed. The first is an update of the risk assessment from D5.8 with the new POD, but based on the same three studies from 2010-2013. The second risk assessment uses the new data from 2014 to 2018 from the aligned studies within HBM4EU.

9.3.2.1 Derivation of the POD

A provisional HBM guidance value was based on a POD, reflecting the critical endpoint identified in hazard characterisation and used as a reference point for the urinary concentration levels measured. For the derivation of the provisional HBM guidance value, the urinary mass balance approach was applied (Apel, 2020). This method can be applied to substances that are primarily eliminated through urinary excretion, and for which regular repeated exposure is likely. For such substances, the approach assumes a balance between the substance intake and the substance excretion, reaching a steady-state in the urine matrix. Under this assumption, the urinary excretion rate is a constant fraction of the intake rate (Angerer et al., 2011).

Using this method, the animal POD was first extrapolated to a human equivalent (external) toxicological reference value (TRV) with application of assessment factors (AF) to account for extrapolation uncertainties (see Equation 1). The factors for allometric scaling and remaining toxicokinetic differences were applied to account for differences in toxicokinetics and toxicodynamics between experimental animals and humans. An intraspecies factor was applied to consider toxicological sensitivity differences between humans due to differences in biology (age, sex, health status, etc.). Default AF values were determined following the consensus in the ECHA guidance chapter R.8 (European Chemicals Agency) (ECHA, 2012).

Equation 1 : $$\mathrm{TRV} = \frac{\text{Animal PoD}}{\text{Overall AF}}$$

Next, a provisional HBM guidance value for the general population was calculated from the TRV, using Equation 2, with parameters defined as follows:

- provisional HBM guidance value (GenPop): the provisional Human Biomonitoring guidance value below which no adverse health effect should be expected in the general population, expressed as the substance concentration per unit of urine volume or creatinine (mg/L urine or mg/g creatinine)
- TRV: toxicological reference value, the external human value corresponding to the animal POD (mg/kg bw/day)
- Fue: substance-specific steady-state fraction of urinary excretion, the daily proportion of the intake dose excreted in urine.
- Daily urine/creatinine excretion rate adjusted to BW: typical 24-hour urine volume or creatinine excreted, adjusted to default human bodyweight (L/kg bw/day or g/kg bw/day)

Equation 2 $$\text{provisional HBM} - \text{GV (GenPop)} = \frac{\text{TRV} * \text{Fue (substance)}}{\text{Daily urine/creatinine excretion rate adjusted to BW}}$$

9.3.2.2 Exposure assessment

9.3.2.2.1 Systematic review

For the purpose of D5.8, a systematic review was performed to assess the levels of BP-3 exposure in the European general population using the search engine Embase.com (Amsterdam: Elsevier) to gather all available Human Biomonitoring studies. Articles were qualitatively included for compiling the BP-UV filter exposure database with European exposure data. Articles were then quantitively screened for meta-analysis of BP-3 exposure in the European general population. To ensure analysis of periods of constant exposure to BP-3 before and after the regulatory amendment (2017), only data after 2006 was included. The end date was 23rd of March 2020.

Studies meeting one of the following exclusion criteria were excluded from the review:

a) non-human studies *(search filter)*;
b) no biomonitoring data;
c) not analysing BP-3;
d) analysing experimental exposure to BP-3;
e) non-European populations;
f) data sampled before 2006;
g) full paper not available;
h) urine samples stored >4°C (to prevent degradation, Vidal et al., 2007);
i) urine samples not enzymatically deconjugated (Gonzalez et al., 2006).

After study selection based on the criteria, the overlapping cohorts amongst the multiple included articles were removed. In filtering out the overlapping cohorts, studies with larger sample size, more detailed data reporting, and analysis of multiple benzophenones, were prioritised. The risk of bias (RoB) of the individual studies was evaluated and studies were excluded from the meta-analysis in case of a considerable RoB.

The summary statistics were calculated for typical case (TC) exposure and reasonable worst-case (RWC) exposure. The typical case exposure is described by the median and range (min-max) of median values from the included studies and the reasonable worst-case by the median and range (min-max) of P95 values. Descriptive statistics that were below LOD or LOQ were replaced with the corresponding LOD or LOQ value, conforming with the statistical analysis of the CONTAM panel (Knutsen et al., 2018). All statistical analyses were performed in Excel.

9.3.2.2.2 Aligned study data

HBM4EU includes cohort studies from various countries in Europe. For the benzophenone risk assessment, aggregated data were collected from the HBM4EU repository. The studies, their region, and age group that included measurements of BP-3 are shown in table 1.

Table 1: overview of aligned HBM studies within HBM4EU including benzophenones

Cohort name	Institute	Country	HBM4EU region	Age group included	Sampling period
German Environmental Survey (GerES) V	UBA	Germany	West	Adults, Teenagers	2016-2018
Oriscav-Lux2	LNS	Luxembourg	West	Adults	2014-2017
Riksmaten adolescents	SEPA	Sweden	North	Teenagers	2015-2017
Norwegian environmental biobank (NEB) II	NIPH	Norway	North	Teenagers	2016-2017
Biomonitorización en adolescentes (BEA)	ISCIII	Spain	South	Teenagers	2017-2018
POLAES	NIOM	Poland	East	Teenagers	2016-2017

All studies measured urine values of both BP-1 and BP-3. In addition, BP-7 (5-chloro-2-hydroxybenzophenone) was determined in the studies from Poland, Spain, and Sweden. As only a POD is available for BP-3, the risk assessment was limited to BP-3. All studies performed urine measurements with creatinine correction, thus creatinine corrected data were used for the risk assessment. More information on the aligned studies within HBM4EU can be found in Gilles et al. 2021.

9.3.2.3 Risk characterisation

The risk characterisation of BP-3 included the calculation of the Risk Characterisation Ratio (RCR) (van Leeuwen & Vermeire, 2007). This ratio was calculated by comparing the TC exposure estimate and the RWC exposure estimate to the provisional HBM guidance value as described in *Equation 3* and *Equation 4*:

$$\textbf{Equation 3: } \mathrm{RCR\ (TC)} = \frac{\text{Range of median values}}{\text{provisional HBM} - \mathrm{GV}}$$

$$\textbf{Equation 4: } \mathrm{RCR\ (RWC)} = \frac{\text{Range of P95 values}}{\text{provisional HBM} - \mathrm{GV}}$$

RCRs greater than 1 implicate that the population assessed is partly exposed above the derived provisional HBM guidance value and is thus potentially at risk of adverse effects from BP-3 exposure.

9.3.3 Results

9.3.3.1 Hazard identification and characterisation

9.3.3.1.1 POD derivation by the SCCS

Under REACH BP-3 is characterised as non-sensitising, non-irritant to eyes and skin, and without indication of acute adverse effects (ECHA, 2020). Both human epidemiological and animal experimental studies have associated BP-3 exposure with reproductive and developmental toxicity (French, 1992; Philippat et al., 2012; Tang et al., 2013). However, BP-3 does not meet the CLP criteria for reproductive toxicity classification (EC 2020). BP-3 exposure has been linked to estrogenic, androgenic, and antiandrogenic activity *in vivo* and *in vitro* (Ghazipura et al., 2017; Kim & Choi, 2014). However, the clinical relevance of these endocrine effects is not always clear, and uncertainty remains over when the observed endocrine effects should be interpreted as adverse. Therefore, the recent updated opinion of the SCCS paid specific attention to the endocrine-disrupting properties of BP-3 (SCCS/1625/20). In this review, SCCS concluded that whilst there are indications from some studies to suggest that BP-3 may have endocrine effects, it is not conclusive enough at present to enable deriving a new ED-related toxicological POD for use in safety assessment. The NOAEL of 67.9 mg/kg bw/day was taken as POD by SCCS from a reproductive toxicity study in rats (Nakamura et al., 2015) and based on a reduction of the number of spermatocytes per seminiferous tubule in offspring at doses of 207.1 mg/kg bw/day and higher. This POD was used to derive the provisional HBM guidance value used as a reference point in the risk assessment. For comparison, the POD previously derived by the SCCS and used in D5.8 was a NOAEL of 200 mg/kg bw/day from a prenatal developmental toxicity study (OECD TG 414) based on urine-stained fur, effects on food consumption and body weight, and decreased net maternal body weight gain in dams, and skeletal variations in the skull and cervical arch structures in the foetuses at 1000 mg/kg bw/day (SCCS/1201/08).

9.3.3.1.2 Provisional HBM guidance value derivation

In accordance with ECHA guidance R.8, assessment factors (AFs) for allometric scaling, remaining inter- and intraspecies differences were applied (ECHA, 2012). The guidance additionally prescribes an AF for exposure duration extrapolation, where appropriate. In this case, as the effect on spermatocytes was observed in the developing pups, and in contrast with the previous POD used in D5.8, no additional AF was applied.

An overall AF of 100 was constituted by the following default AFs:

- Allometric scaling (rat to human): 4
- Remaining interspecies differences: 2.5
- Intraspecies differences: 10
- Duration extrapolation: 1
- *Overall AF:* *4 * 2.5 * 10 *1 =* *100*

The F_{ue} was obtained from two human experimental exposure studies (Hayden et al., 1997; Sarveiya et al., 2004). Hayden et al. (1997) report a BP-3 output in urine of 1-2% of the initial dose, 10 hours after initial dermal application to nine healthy adult volunteers. Sarveiya et al. (2005) similarly report up to 1% BP-3 of the initial dose dermally applied to three female volunteers, measured in urine after 48 hours. It was decided to incorporate a F_{ue} of 0.01 (1%) as the more conservative value. As there is no reliable dermal toxicity study is available nor a conversion factor from oral to dermal, it had to be assumed that absorption and metabolism are the same in both routes.

The HBM4EU default average daily creatinine excretion was incorporated in the derivation of the creatinine corrected provisional HBM guidance value. The HBM4EU default creatinine value is based on the calculation in (Aylward et al., 2009) and amounts to an average creatinine excretion of 1.4 g/L per day for both sexes combined. Adjusted to the default bodyweight of 70 kg, as defined by ECHA R.8 and followed by HBM4EU, this amounted to an average creatinine excretion rate of 0.02 g/kg bw/day.

Table 2 presents an overview of the information and parameters incorporated in the provisional HBM guidance value calculation.

Table 2: Overview of parameter values incorporated in the provisional HBM guidance value calculation, with sources

Parameter	Value	Source
Animal PoD	NOAEL = 67.9 mg/kg bw/day	SCCS opinion (2021)
Overall AF	AF = 100	ECHA R.8 guidance
F_{ue}	1% or 0.01	Hayden et al. (1997); Sarveiya et al. (2005)
24h creatinine	0.02 g/kg bw/day	HBM4EU default from Aylward et al. (2009)

Application of the overall AF to the animal PoD in *Equation 1* provided the TRV:

$$\mathrm{TRV} = \frac{67.9}{100} = 0.68\ \mathrm{mg/kg\ bw/day}$$

Incorporating the TRV and remaining parameters (Table 2) in *Equation 2* provided the provisional HBM guidance value:

$$\mathrm{HBM-OV\ (GenPop)} = \frac{0.68 * 0.01}{0.02} = 0.34\ \mathrm{mg/g\ creatine} = 340\ \mu\mathrm{g/g\ creatinine}$$

9.3.3.2 Exposure and risk assessment

9.3.3.2.1 Systemic review

Exposure was assessed with a meta-analysis of the biomonitoring data. Screening and selection resulted in 17 articles with biomonitoring data on benzophenone-3 in European cohorts. After application of the exclusion criteria and selection of the studies that used sufficient numbers of samples (>120) 8 studies were selected. To be able to compare the outcomes, only the 3 studies that used creatinine correction were included in the meta-analysis (Adoamnei et al. 2018; Dewalque et al. 2014; Frederiksen et al. 2013). Table 3 shows the metadata from these three studies.

Table 3: Studies included (final total of three studies) per European region as defined by HBM4EU with corresponding information on sample size, sex, and age range (minimum-maximum age in years)

European region	Studies	Samples	Sex	Age range
North	N = 1	N = 288	M/F	6 - 52
West	N = 1	N = 261	M/F	1 - 85
South	N = 1	N = 215	M	18 - 23
East	NA	NA	NA	NA

Table 4 presents the cohort characteristics and study-specific descriptive statistics of creatinine-corrected concentrations of BP-3 and BP-1. Frederiksen et al. (2013) reported BP-3 measurements in first morning urine samples of mother-child pairs in Denmark. Dewalque et al. (2014) reported BP-3 measurements in spot urine samples from the general population in Belgium. Adoamnei et al. (2018) reported BP-3 and BP-1 measurements in first morning urine samples of male students in Spain. Adoamnei et al. (2018) did not report maximum measured concentrations. The typical case BP-3 exposure (based on median values across the studies) ranged from 0.60 to 4.40 μg/g creatinine, with a median of 1.30 μg/g creatinine. The reasonable worst-case BP-3 exposure (based on P95 values across the studies) varied between 16.30 and 392.00 μg/g creatinine, with a median of 33.00 μg/g creatinine.

Table 4: Cohort characteristics and descriptive statistics reported by the final three studies included in the meta-analysis of exposure. All concentrations (P50, P75, P95, max) are creatinine-corrected and thus expressed as μg/g creatinine. Concentrations > provisional HBM guidance value in bold. (N: sample size, M: male; F: female; age range: minimum-maximum age in years; LOD: limit of detection; NA: not available; RCR: Risk Characterisation Ratio)

Study	Country	Sampling period	N/sex	Age range	Sample	Chemical	LOD (% > LOD)	P50	P75	P95 (RCR)	Max
Frederiksen et al. (2013)	Denmark	09/2011 - 12/2011	143 M/F	6 - 11	Morning	BP-3	0.07 (97.0%)	2.00	6.30	33.00 (0.1)	408.00
			154 F	31 - 52	Morning	BP-3	0.07 (98.0%)	4.40	15.00	392.00 (1.15)	2139.00
Dewalque et al. (2014)	Belgium	01/2013 - 04/2013	123 M	2 - 75	Spot	BP-3	0.20 (82.1%)	0.60	2.00	28.80 (0.08)	414.20
			138 F	1 - 85	Spot	BP-3	0.20 (83.3%)	1.30	4.40	33.30 (0.1)	141.30
Adoamnei et al. (2018)	Spain	10/2010 - 11/2011	215 M	18 - 23	Morning	BP-3	0.20 (65.6%)	0.96	4.60	16.30 (0.05)	NA

Comparing the typical case (TC) exposure range (0.60 – 4.40 µg/g creatinine) and the reasonable worst-case (RWC) exposure range (16.30 – 392.00 µg/g creatinine) with the provisional HBM guidance value (340 µg/g creatinine) in *Equation 3* and *Equation 4*, provided the following RCRs:

$$\text{RCR (TC)} = \frac{0.60 \text{ to } 4.40}{340} = 0.002 \; to \; 0.01 = RCR < 1$$

$$\text{RCR (RWC)} = \frac{16.30 \text{ to } 392.00}{340} = 0.05 \; to \; 1.15 = RCR > 1$$

For typical case exposure, the RCR was below 1, so median HBM results did not exceed the provisional HBM guidance value. For reasonable worst-case exposure, the RCR slightly exceeded 1. Table 4 shows the HBM values exceeding the provisional HBM guidance value in bold, as well as the RCR values at the RWC/P95 value. The P95 value reported by Frederiksen et al. (2013) for Danish females exceeded the provisional HBM guidance value. The maximum values for female participants and children in Frederiksen et al. (2013), and for male participants in Dewalque et al. (2014), exceeded the provisional HBM guidance value.

In summary, in the worst case exposure scenario, HBM values slightly exceeded the provisional HBM guidance value, indicating potential risk to the heavily exposed part of the population. Typical exposure levels however, were within safe limits.

9.3.3.2.2 Aligned study data

Benzophenone UV-filters were determined in six studies aligned within HBM4EU from 2014 to 2018. Five studies performed measurements in teenagers and two in adults. Both males and females were included in every study. The sampling time varied between 24 hr samples, spot samples and first morning urine. When samples were below the LOD/LOQ, the LOD/2 or LOQ/2 was used.

An overview of the results is presented in table 5. The typical case BP-3 exposure based on the P50 ranged from <LOQ to 3.68 µg/g creatinine. The reasonable worst case exposure, based on the P95, ranged from 18.97 to 68.83 µg/g creatinine.

Comparing the typical case (TC) exposure range and the reasonable worst-case (RWC) exposure range with the provisional HBM guidance value (340 µg/g creatinine) in *Equation 3* and *Equation 4*, provided the following RCRs:

$$\text{RCR (TC)} = \frac{- \text{ to } 3.68}{340} = -to \; 0.01 = RCR < 1$$

$$\text{RCR (RWC)} = \frac{21.62 \text{ to } 68.83}{340} = 0.06 \; to \; 0.20 = RCR < 1$$

Table 5: Overview of the outcome of the BP-3 measurements in the aligned studies in µg/g creatinine and the RCRs of the P50 and P95 calculated with the provisional HBM guidance value of 340 µg/g creatinine. The results of all studies included both male and female participants.

Country	Sampling period	N	Age range	Sample	% above LOD/ LOQ	P05	P10	P25	P50	P75	P90	P95	RCR P50	RCR P95
Luxembourg	2016-2018	210	25-39	Spot	99.51 %	0.13	0.17	0.44	0.94	2.64	10.21	18.97	0.00	0.06
Germany	2014-2017	180	20-29	24 hr	30%	<LOQ	<LOQ	<LOQ	<LOQ	3.00	11.25	27.52	-	0.08
-	2015-2017	56	12-18	Morning	30.36 %	<LOQ	<LOQ	<LOQ	<LOQ	3.25	7.10	21.62	-	0.06
Norway	2016-2017	181	12-14	Spot	100%	0.66	0.80	1.55	3.50	8.25	28.84	55.92	0.01	0.16
Poland	2017	249	12-14	Spot	100%	0.59	0.76	1.40	3.68	9.52	34.97	67.22	0.01	0.20
Sweden	2016-2017	300	12-17	Spot	94.33 %	<LOD	0.11	0.29	0.88	2.64	11.31	33.87	0.003	0.1
Spain	2017-2018	299	13-17	Spot	99%	0.46	0.65	1.45	3.01	7.20	30.25	68.83	0.009	0.20

BP-3 was detected in relatively low levels in all countries investigated. Due to differences in LOQ/LOD, the rate of detection differed between studies. In both the typical case and reasonable worst case scenarios the exposure did not exceed the provisional HBM guidance value and no risk was indicated.

9.3.4 Discussion

In the previous report (HBM4EU D5.8), a risk assessment was presented of BP-3 on biomonitoring data from three studies performed in 2010-2013, which has been included here with the new POD from the latest SCCS opinion. The outcome showed that the exposure of the majority of study participants did not give rise to concern, while a potential risk could occur in highly exposed groups. In this evaluation a lower POD was used, but also a lower AF due to the difference in endpoint which merited dropping the time factor. As the difference in the resulting provisional HBM guidance value was relatively small between the previous deliverable and this one (less than 3-fold decrease), the outcome of the risk assessment stayed essentially the same.

Several of the aligned studies conducted from 2014 to 2018 within HBM4EU performed new measurements of benzophenones, including BP-3. The question was whether there would be any change in the exposure, in particular due to the lower maximum allowed concentration of BP-3 in sunscreen products.

9.3.4.1 The influence of time, place, age, and sex on BP-3 exposure

When comparing the results of the previous assessment and aligned studies, the most notable difference between the outcomes is that indeed the P50 and P95 are lower in the more recent measurements. Of the six studies the highest RCR at the P95 was 0.2, where it was 1.15 in the

previous assessment. This seems to indicate that exposure to BP-3 decreased over the past years. However, without disregarding this finding, there are a few nuances that should be made.

The first and foremost is that the high P95 value from the literature was limited to one group of females from a Danish study, which was ten times above the P95 values of the other groups. In fact, the other P95 values are comparable with the P95 values from the aligned studies. Unfortunately, no Danish cohort was included in the aligned studies, as this would have allowed a more meaningful comparison over time.

Only Spanish cohorts were included in both groups of studies and here higher values were found in the new study, albeit still well below the provisional HBM guidance value. This is probably related to the different population included in the cohorts. In the study by Adoamnei et al. (2018), only male students were included, while the aligned study included both male and female teenagers.

Previous research reported higher BP-3 levels in females compared to males (Gao et al., 2015; Sakhi et al., 2018). Also in the aligned studies the BP-3 levels were higher in females than in males, as can be seen in Appendix A. In particular in two studies, the levels in females were markedly higher with P95 values of 120 and 207 µg/g creatinine. At these levels, the RCRs are still below 1, namely 0.35 and 0.61, indicating no increased risk. However, this outcome does confirm that BP-3 exposure sometimes differs between sexes.

This raises the question which products contribute to BP-3 exposure. Studies on BP-3 exposure sources report frequent occurrence and high concentrations of BP-3 in cosmetic products, such as sunscreens, skin lotions, foundations, and lip and eye make-up (DEPA, 2015; Ko et al., 2016; Liao & Kannan, 2014). Self-reported sunscreen use has been strongly associated with BP-3 concentrations in urine (Zamoiski et al., 2015). While sources other than sunscreen lotions and other cosmetics have been identified as well, based on content and frequency of use, these are estimated to contribute only 10% of the exposure to BP-3 (DEPA, 2015).

With regard to age, most of the aligned studies measured benzophenones only in teenagers and only the German and Luxembourg studies included (relatively young) adults. No notable differences were found between these groups. As the highest exposure in previous studies was found in age ranges above those included in the aligned studies, one should be careful with the extrapolation of these results to the entire population.

Although there were differences in sampling time (24 hr, spot or morning samples), there is no clear effect of this to be seen in the resulting values. Both in the literature and aligned studies, the highest and lowest values were both found in morning urine samples.

9.3.4.2 Other benzophenone UV-filters

In the aligned studies also other benzophenone UV-filters were measured in addition to BP-3. Out of six studies, five determined BP-1 and four also BP-2 and BP-7. The aggregated results of all benzophenone measurements can be found in Appendix B.

As there are no Guidance Values for the other benzophenones besides BP-3, no risk assessment was performed for these compounds. However, when considering the results it is clear that the exposure to BP-2 and BP-7 was very limited. In the four studies that included them, BP-2 and BP-7 were found in less than 10% of urine samples and the detected concentrations were low. BP-1 was found with approximately the same frequency as BP-3, albeit at lower levels in all five studies that included it. The relative concentration of BP-1 was approximately 1/3 to 1/4 compared to BP-3. As BP-1 is a metabolite of BP-3 this finding is not unexpected. It would be interesting to know whether the participants also use BP-1 based sunscreen products, or whether indeed all BP-1 measured

was a metabolite of BP-3. The latter seems more likely considering the lower levels and product use investigations that found that BP-1 occurs as a parent compound in much smaller amounts relative to BP-3 (DEPA, 2015). Unfortunately, the fraction of BP-3 that is converted to BP-1 could not be derived from the dermal absorption studies in humans.

9.3.4.3 Provisional guidance value derivation and risk assessment

The provisional guidance value derivation was based on the POD from the SCCS opinion (SCCS, 2021). The POD was based on a reduction of the number of spermatocytes per seminiferous tubule in offspring after exposure of the mothers at higher doses. As this effect is subject to a critical window of exposure, namely during pregnancy of the mothers, the AF for exposure duration extrapolation is less relevant (ECHA, 2012). This is different from the POD from the previous SCCS opinion, which was used in D5.8. There an AF of 3 was applied for exposure duration as the effect was observed in the mothers as well.

The provisional guidance value derivation method included some assumptions. The POD is derived from an oral study in rats, while the F_{ue} is based on dermal absorption in humans. Also the most important route of exposure to BP-3 in real life is likely to be dermal. As there is no reliable dermal toxicity study suitable for the derivation of a point of departure, nor an appropriate study that compares both routes, it is assumed that the absorption and metabolism are the same in both routes. However, in the SCCS opinion (2021) a dermal adsorption value of 9.9% was used based on *in vitro* skin penetration experiments. As the dermal absorption is expected to be lower than via the oral route, the assumption of equal uptake will probably result in a more conservative guidance value and an overestimation of the risk.

The urinary mass balance approach assumes a balance between substance intake and substance excretion and, accordingly, derives the provisional HBM guidance value by application of the F_{ue} as a steady-state metabolic conversion factor (Angerer et al., 2011). In reality, substance excretion may vary based on intake-rate and half-life elimination. The limited toxicokinetic information from both animal and human studies does not point to rapid elimination from the body, which creates uncertainty in the F_{ue} value. Rat studies show biphasic elimination with alpha half-life 0.88 – 1.3 hours and beta half-life 15.05 – 15.90 hours (Kadry et al., 1995; Okereke et al., 1994). Matta et al. (2019) report a long terminal half-life in humans with a mean range of 24-31 hours for BP-3. The detection of BP-3 in human breast milk and adipose tissue might suggest possible build-up of BP-3 in humans (Schlecht et al., 2004; Wang et al., 2015). More substance-specific toxicokinetic data is required to improve the reliability of the F_{ue} including data on blood levels over time. Compared to several other chemical substances, the current F_{ue} seemed to be on the low side (Asimakopoulos et al., 2014). Asimakopulous et al. (2014) incorporated a F_{ue} of 2%, based on Hayden et al. (1997), in the transformation of internal estimates to external intake estimates. As the current study transformed external intake estimates to internal exposure levels, a F_{ue} of 0.01 (1%) was considered the more conservative value. In case the F_{ue} was in reality higher, the current provisional HBM guidance value value is slightly overestimating the risk ratio.

Generalising the provisional HBM guidance value to the general European population should be done with caution. The F_{ue} was obtained from small groups and is thus likely not representative of the general population (Angerer et al., 2011). Daily creatinine excretion rates are also subject to interindividual toxicokinetics variations. The default value from Aylward et al. (2009) represents a predominantly male group of participants with an acceptable default bodyweight of 70 kg assumed. Incorporation of this default value in the BeV derivation likely affected the generalisability of the provisional HBM guidance value for the general population. Improved default creatinine excretion values are considered within HBM4EU WP5. While interindividual variation in the F_{ue} and typical creatinine values might affect the representativeness, these variations were partly accounted for by

application of the assessment factors. Besides, these uncertainties are urine matrix specific and do not make HBM data unusable. More extensive knowledge on the F_{ue} and average creatinine excretion values in different population groups might lower the intraspecies AF and further refine the risk assessment.

In contrast to the latest SCCS Opinion (SCCS 2021) a risk for European population could not be identified in the risk assessment based on recent HBM data. The SCCS concluded that the current maximal BP-3 concentration of 6 % in sunscreen products is not safe for consumers and suggested reducing the maximal concentration to 2.2 %. According to the data from the aligned HBM studies and the risk assessment conducted here, one might conclude that the BP-3 reduction might not be necessary. However, the SCCS assumes concurrent exposure from all cosmetic product types, while such a scenario may not or only rarely have occurred in the participants of the HBM studies. In the absence of information about sunscreen use, it is unknown whether peak exposure might be higher than measured in the aligned studies. Depending on the actual sunscreen use in the HBM study participants, it should also be debated whether the P95 is truly a RWC scenario, or that a P97 or even max value would be a better representative of heavy sunscreen users. In summary, this example shows the value of HBM data in risk assessment and possibly resulting regulatory consequences, but also the importance of information on exposure sources if HBM data is indeed to be used in regulatory contexts.

9.3.4.4 Implications for future research

The risk assessment of BP-3 could be further refined by more substance-specific toxicokinetic research. Further toxicokinetic evidence on BP-3 metabolism and elimination might improve the reliability of the F_{ue} and allow for more precise consideration of metabolites. Moreover, if stronger evidence arises that this substance has the potential to accumulate in humans, it may be worthwhile to study exposure in another matrix such as the blood. Incorporation of HBM data on BP-3 metabolites could improve the precision of the BP-3 exposure assessment. Furthermore, methods should be developed to study possible mixture effects of exposure to BPs that need to be included in the assessment besides BP-3's metabolites (i.e. BP-1).

More detailed research on exposure sources of BP-3 would benefit the relevance of HBM data in risk management. The advantage of HBM data is that actual aggregated exposure is determined, but for risk management also insight in exposure sources is needed to know if and to what extent the study population was exposed to the product that is being regulated.

The outcome of the studies shows that HBM data can provide useful insights in identifying highly exposed groups. Time-trend analysis based on HBM can quantify the actual population-wide effects of specific policy changes. By giving insight in total exposure from all sources and routes, HBM can be a valuable aid in the one substance one assessment goal of the new Chemical Strategy for Sustainability, in particular for substances as BP-3 which falls under multiple regulations.

9.3.5 Conclusion

In conclusion, this preliminary risk assessment of BP-3 implied that in general there is no risk to consumers expected. It also shows that there are notable differences in exposure between groups, with the most highly exposed groups approaching the acceptable risk levels for BP-3, within one order of magnitude of exceedance (but without surpassing this level). This analysis was greatly aided by the coordination of HBM studies within HBM4EU, which ensured that measurements were performed and analysed in a standardised way that enabled a meaningful comparison. This study notes the value of empirical HBM data and highlights the importance of the HBM4EU initiative in advancing and coordinating biomonitoring research in Europe.

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9.5 Appendix A: Aggregated data for BP-3 stratified by sex (in µg/g creatinine)

Country	Sampling period	N	Age range	Sample	Sex	% above LOD/LOQ	P05[1]	P10	P25	P50	P75	P90	P95
Luxembourg	2016-2018	95	25-39	Spot	M	98.95	0,09	0,14	0,39	0,75	1,81	3,52	6,61
		108			F	100	0,17	0,22	0,50	1,45	3,67	15,69	30,79
Germany	2014-2017	90	20-29	24 hr	M	23.33	-3	-3	-3	-3	-3	5,35	10,93
		90			F	36.67	-3	-3	-3	-3	6,41	17,11	48,26
-[1]	2015-2017	24	12-18	Morning	M								
		32			F								
Norway	2016-2017	77	12-14	Spot	M	100	0,59	0,71	1,16	2,16	5,79	24,89	48,27
		104			F	100	0,81	1,04	2,22	4,40	9,59	28,23	54,31
Poland	2017	140	12-14	Spot	M	100	0,53	0,68	1,03	2,99	8,11	22,51	37,75
		109			F	100	0,82	1.00	1,84	4,84	15,20	56,53	207,26
Sweden	2016-2017	150	12-17	Spot	M	92.67	-1	0,07	0,21	0,61	1,82	5,39	15,95
		150			F	96	0,08	0,23	0,44	1,40	3,88	25,74	41,56
Spain	2017-2018	143	13-17	Spot	M	100	0,40	0,53	1,13	2,25	5,10	9,71	41,06
		156			F	98.08	0,61	0,91	1,77	3,81	10,81	45,23	119,63

[1]No data given as N<50

9.6 Appendix B: Aggregated data of all benzophenone UV-filters from the aligned studies (in µg/g creatinine)

Country	Sampling period	N	Age range	Sample	Chemical	% above LOD/LOQ	P05[1]	P10	P25	P50	P75	P90	P95
Luxembourg	2016-2018	210	25-39	Spot	BP-3	99.51%	0,13	0,17	0,44	0,94	2,64	10,21	18,97
					BP-1	100%	0,04	0,07	0,16	0,37	1,06	3,03	6,85
					BP-2	2.96%	-1	-1	-1	-1	-1	-1	-1
					BP-7	3.94%	-1	-1	-1	-1	-1	-1	-1
Germany	2014-2017	180	20-29	24 hr	BP-3	30%	-3	-3	-3	-3	3,00	11,25	27,52
					BP-1	30%	-3	-3	-3	-3	0,82	3,12	7,34
-	2015-2017	56	12-18	Morning	BP-3	30.36%	-3	-3	-3	-3	3,25	7,10	21,62
					BP-1	44.64%	-3	-3	-3	-3	0,98	2,27	6,90
Norway	2016-2017	181	12-14	Spot	BP-3	100%	0,66	0,80	1,55	3,50	8,25	28,84	55,92
Poland	2017	249	12-14	Spot	BP-3	100%	0,59	0,76	1,40	3,68	9,52	34,97	67,22
					BP-1	100%	0,22	0,31	0,67	1,66	4,57	16,35	26,35
					BP-2	6.43%	-1	-1	-1	-1	-1	-1	0,05
					BP-7	4.8%	-1	-1	-1	-1	-1	-1	-1
Sweden	2016-2017	300	12-17	Spot	BP-3	94.33%	<LOD	0,11	0,29	0,88	2,64	11,31	33,87
					BP-1	97.33%	0,05	0,07	0,17	0,47	1,70	5,64	12,53
					BP-2	4%	-1	-1	-1	-1	-1	-1	-1
					BP-7	7.67%	-1	-1	-1	-1	-1	-1	0,13
Spain	2017-2018	299	13-17	Spot	BP-3	99%	0,46	0,65	1,45	3,01	7,20	30,25	68,83
					BP-1	100%	0,19	0,32	0,66	1,33	3,33	12,78	23,29
					BP-2	5.69%	-3	-3	-3	-3	-3	-3	0,05
					BP-7	3.34%	-3	-3	-3	-3	-3	-3	-3

[1]The value -1 indicates X < LOD, the value -3 indicates X < LOQ