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2 Glossary

ADI	Acceptable Daily Intake
ABD	Attributable Burden of Disease
AOPs	Adverse Outcome Pathways
BaP	Benzo[a]pyrene
BBP	Benzyl butyl phthalate
BE	Biomonitoring Equivalent
BGV	Biological Guidance Value
BLV	Biological Limit Value
BMD	Benchmark Dose
BMDL	Benchmark Dose Lower Confidence Limit
BoD	Burden of Disease
BOELV	Binding Occupational Exposure Limit Value
CDC	Center for Disease Control
CKD	Chronic Kidney Disease
Cr(VI)	Hexavalent Chromium
DBP	Dibutyl phthalate
DEHP	Bis(2-ethylhexyl) phthalate
DIBP	Diisobutyl phthalate
DIDP	Diisodecyl phthalate
DINP	Diisononyl phthalate
DINCH	Diisononyl cyclohexane-1,2-dicarboxylate
DMP	Dimethyl phthalate
DNEL	Derived No-Effect Level
DPHP	Di(2-propylheptyl)phthalate
ECHA	European Chemicals Agency
EFSA	European Food Safety Authority
EU	European Union
FIOH	Finnish Institute of Occupational Health
GV	Guidance Value
HBGV	Health-Based Guidance Value
HBM	Human Biomonitoring
HBM-GV	Human Biomonitoring Guidance Value
HBMI	European Human Biomonitoring Initiative

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HIA	Health Impact Assessment
IARC	International Agency for Research on Cancer
IPCheM	Information Platform for Chemical Monitoring
IPCS	International Programme on Chemical Safety
JRC	Joint Research Center
KE	Key event
LOAEL	Lowest Observed Adverse Effect Level
MoA	Mode of Action
MOCA	4,4'-Methylene-bis-(2-chloro-aniline)
MoS	Margin of Safety
NO(A)EL	No Observed (Adverse) Effect Level
OECD	Organisation for Economic Co-operation and Development
OEL	Occupational Exposure Limit
PAHs	Polycyclic Aromatic Hydrocarbons
PBPK/PBTK	Physiologically Based Pharmacokinetic/Toxicokinetic
PBDEs	Polybrominated diphenyl ethers
PBT	Persistent Bioaccumulative and Toxic
PCBs	Polychlorobiphenyls
PFAS	Per- and polyfluoroalkyl substances
PFCs	Perfluorinated Compounds
PFHxS	Perfluorohexanoic Sulphonic Acid
PFNA	Perfluorononanoic Acid
PFO	Perflurooctanoate
PFOA	Perfluorooctanoic Acid or Pentadecafluorooctanoic acid
PFOS	Perfluorooctane Sulfonic Acid or Heptadecafluorooctane-1-sulphonic acid
POD	Point of Departure
RA	Risk assessment
RAC	Risk Assessment Committee
RAR	EU Risk Assessment Report (pre-REACH)
RCR	Risk Characterization Ratio
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
RfD	Oral Reference Dose (US EPA)
RMM	Risk Management Measures

RV	Reference Value
SCOEL	Scientific Committee on Occupational Exposure Limits
SEAC	Socio-Economic Analysis Committee
SVHC	Substance of Very High Concern
TDI	Tolerable Daily Intake
TWA	Time-weighted average
TWI	Tolerable Weekly Intake
WP	Work Package
WHO	World Health Organization

3 Abstract/Summary

The aim of this work was to exemplify the inclusion of human biomonitoring (HBM) data in risk assessment (RA) and health impact assessment (HIA) strategies. RA was performed for six compound groups on HBM4EU's first list of priority substances: anilines, cadmium/chromium, flame retardants, PAHs, PFAS and phthalates. In addition, burden of disease (BoD) calculations were made for cadmium.

The general approach used included: 1) identification of an existing RA for the substance, 2) identification of possible existing biological limit or guidance values or biological equivalents (BEs), or if lacking, existing health based limit values for external exposure, 3) identification of relevant biomonitoring data to be used in the RA, 4) in case no existing biological limit or guidance values or BEs existed, identification of approaches for reverse/forward calculation, including the use of PBPK modelling or calculation of BE values based on one-compartment modelling, 5) RA or BoD calculation based on HBM data, 6) analysing the benefits and challenges of using HBM data in RA compared to the use of external exposure data.

The overall result of the work was that HBM can be included in RA even when relatively few data are available, and its inclusion generally benefits the RA. Several methods exist, and a tiered approach is suggested, based on the amount and quality of data available. The recommended 1st tier method is a one-compartment modelling based derivation of BE values or reverse calculation of external exposure based on biomarker levels. This approach is simple and rough, and uses only very basic parameters. However, in many cases this approach can be considered sufficient, especially when conservative assumptions have been used for the F_{UE}, and the calculated RCRs remain well below 1, indicating a low risk. Also, in cases in which risk assessment using this approach supports the RA made based on external exposure estimates, it is often a sufficient approach. Nevertheless, in some cases e.g. where the RCR is close to 1, a more detailed approach may be needed to refine the RA. For the 2nd tier, PBPK modelling is recommended. For the most robust, 3rd tier approach, measured data on correlations between external exposure and internal doses from well controlled studies would be needed.

Certain cases were identified where inclusion of HBM would be particularly important for performing RA: for compounds, for which several exposure routes may contribute to the body burden and the health effects, as HBM reflects the total body burden, and cumulative compounds. For cumulative compounds, HBM could also be useful for hazard assessment in addition to exposure assessment. One of the major challenges for the inclusion of HBM into RA is the often limited data available on toxicokinetics. In addition, in some cases, there is an urgent need for more specific biomarkers or more sensitive analytic methods than currently available.

It should be noted that these risk assessments were performed purely to determine how HBM data can contribute to the risk assessment of chemicals, and they have no regulatory implications. Overall for the substances on the HBM4EU's first list of priority substances, more HBM data are needed. This work is ongoing in WP8, and the RAs presented here will be updated when new data become available.

These risk assessments have been performed within HBM4EU to determine how HBM data can contribute to the risk assessment of chemicals. The outcome of these risk assessments has no regulatory implications.

4 Introduction

The objective of task 5.3 in HBM4EU is to perform sample risk assessments (RAs) for priority compounds to demonstrate how human biomonitoring (HBM) can be effectively used in chemical RA. This includes also the use of available HBM guidance values and HBM reference values (GVs and RVs) in RA and health impact assessment (HIA) strategies. In the first year of the project, the current use of HBM in chemicals RA was evaluated (Deliverable Report D 5.1 Human biomonitoring in risk assessment: analysis of the current practice and 1st examples on the use of HBM in risk assessments of HBM4EU priority chemicals). This included a review of RA schemes and a survey for national regulatory risk assessors. Information was collected on their RA practices, use of HBM, and viewed obstacles and challenges related to the use of HBM. Furthermore, examples on the advanced use of HBM under different regulatory RA frameworks were collected on HBM4EU's 1st list of priority substances.

The main conclusions of the first-year work were that while HBM is generally considered as a useful tool, it is mainly seen as 3rd tier method for refinement of exposure assessment. The guidance on the use of HBM in RA is currently generally either limited or completely missing, hence limiting its use. In addition, health based biological limit/guidance values (or biological equivalents, BEs) are considered extremely important for the use of HBM in all RA fields, but especially the lack or paucity of toxicokinetic data may hamper their derivation, even if the toxicological database would otherwise be robust enough. Moreover, the possibilities of employing effect markers in hazard and dose-response analysis appears to mostly not be recognised, possibly due to the uncertainties related to HBM dosimetry and setting relevant dose descriptors, and lack of guidance and knowledge on the meaning and predictive value of different effect markers. Based on the results, some proposals were given for better inclusion of HBM in human RA and HIA. They included collecting and creating successful examples on the use of HBM, to be used as a basis for developing a harmonised guidance on the use of HBM in RA and HIA.

To build on this work, in the second and beginning of the third year of the project, RAs for compounds on the HBM4EU's 1st priority list of chemical groups were performed based on HBM data. These RAs are included as annexes in this report. The aim of this work was to exemplify how HBM data can be used in RA and demonstrate the benefits and challenges in the use of HBM knowledge, including HBM measurements and HBM guidance values (HBM-GVs). Therefore, they are not intended to have regulatory implications, but to rather represent primary attempts with the aim of demonstrating how HBM can be used. In most cases, the RAs presented here will be updated when new data generated within HBM4EU will be available.

5 Approaches used to include HBM data into risk assessment

5.1 Risk assessment and health impact assessment approaches

The general approach for the RAs agreed to be followed was:

1) Identification of the exiting RAs for the substance
2) Identification of existing biological limit or guidance values or BEs, and if not available, existing health based limit values for external exposure
3) Identification of relevant biomonitoring data to be used in RA
4) If there were no existing biological limit or guidance values or BEs, identification of approaches for reverse/forward calculation, including the use of PBPK modelling or one-compartment modelling based calculation of BE values (Angerer et al., 2011)
5) Performing RA/HIA based on biomonitoring data
6) Analysing benefits and challenges of the use of HBM in RA when compared to the use of external exposure data

Full HIA were not made but burden of disease (BoD) calculations were made for cadmium, focusing on osteoporosis and hip and spine fractures.

5.2 GVs and HBM data used in the risk assessments

For the most part, HBM4EU derived HBM-GVs and HBM RVs were not available for the compounds on the 1st list of priority substances. Therefore, different approaches were used in the RAs, according to available data. These included the biomonitoring equivalent (BE) approach, PBPK modelling, and published information on correlations between internal and external doses (table 1). Furthermore, there was a delay in the availability of HBM data in the HBM4EU Data repository and IPCHEM (pre-HBM4EU and HBM4EU generated). Thus, the RA were mainly based on published data. When relevant, it was indicated whether new HBM data generated within HBM4EU could be used to refine these first RAs later.

Table 1: Summary of the substances included in the RAs, and general information on the basis for the exposure and hazard assessments

Substance Group	Specific substances included in the RA	Population covered	Exposure assessment: HBM exposure data used	Hazard assessment: GV used / method for its derivation / other type of method used
Anilines	*ortho*-toluidine	Occupational	Published data from literature	Dose response for carcinogenicity by SCOEL and BE approach to convert biomonitoring data as external intake.
Cd/Cr	Cadmium	General	DEMOCOPHES + published data from literature	Risk assessment (comparison external (based on EFSA, 2009) and internal exposure) and environmental burden of disease calculation (osteoporosis and chronic kidney disease)
	Chromium VI	Occupational	FIOH occupational HBM data (U-Cr) converted into corresponding air levels for exposure assessment	Dose response equation for lung cancer risk (Seidler *et al.*, 2013). Use of existing occupational data on the correlations between air and U-Cr levels.
Flame retardants	TCEP	General	German data from scientific paper (Schindler et al., 2009) as used in AD12.5	External exposure reconstruction (EER) taken from AD12.5 followed by • Calculation of RCR based on US chronic p-RfD[1]: RCR = EER/p-RfD • MOS compared to LOAEL from EU RAR: MOS = EER / LOAEL
PAHs	PYR, BaP, PAH4, PAH8	General	Published data (FLEHS, literature)	Dose response for carcinogenicity (several types of cancer, including lung cancer) by RAC
	PYR, BaP	Occupational	Published data from literature.	Dose response for carcinogenicity (lung, bladder, skin) by RAC
PFAS	PFOA, PFOS, PFNA, PFHxS	General	Published data (FLEHS, literature)	For PFOA and PFOS: cholesterol increase in the human population. For PFNA and PFHxS: hazard data based on animal data
Phthalates	DEHP, BBP, DBP, DIBP, DEP, DMP, DiNP, DiDP, DPHP	General	DEMOCOPHES data, and recent HBM available in the public literature, including German, Danish, and Belgian studies	Dose response for reproductive toxicity effects by RAC. HBM I GV values derived for DEHP and DINCH in task 5.2
	DiNP, DiDP, DPHP	Occupational	FIOH occupational biomonitoring data and published data from literature.	DNELs derived for DINP and DIDP, calculation of BEs corresponding these DNELs. Provisional HBM I GV from task 5.2 (still under revision) used as indicative for DPHP.

[1] p-RfD: US EPA Provisional Oral Reference Dose (an oral guidance value)

6 Summaries of the risk assessments performed

In this chapter, brief summaries of the performed RAs and their main conclusions are provided. For more detailed information, please refer to the complete RA reports, included as annexes at the end of this deliverable.

6.1 Anilines risk assessment

Ortho-toluidine (CAS 95-53-4) is an aniline derivative which is considered to be an animal and human carcinogen, and may cause methemoglobinemia. o-Toluidine is used as a curing agent in epoxy resins and as intermediate in producing herbicides, dyes, and rubber chemicals. It is listed in the candidate list of substances of very high concern for authorization under REACH. A RA was performed by utilizing HBM data (**Annex A**), since the possible health risks should be monitored, especially for workers.

After hazard characterization and exposure assessment, a literature search was conducted on studies concerning o-toluidine HBM data. One-compartment model based approach (Angerer et al., 2011) was used to estimate the urinary levels corresponding to the external intake levels or vice versa. This allowed the comparison between available HBM data and existing binding occupational exposure level (OEL) and established cancer risk estimates.

The existing cancer RA resulted in a Benchmark Dose causing 10% urinary bladder tumour incidence above background level (BMD_{10}) of 42.2 mg/kg bw/day in rats, corresponding to an inhaled dose scaled to humans of 210 mg/m^3 at occupational exposure. Using the approach described above, this level corresponds to a urinary level of 1000 mg/L by assuming a 70-kg bw, a 1.5 L/day urinary volume and 75% excretion. Similarly, for example cancer risk level of 1 : 10 000 corresponds approximately to a steady state urinary level of 1 mg/l.

In conclusion, by applying the BE methodology and based on HBM studies, the workers exposed to o-toluidine have a cancer risk of 1:20 000 in the worst-case scenario (0.5 mg/L in urine). The exposure levels calculated based on HBM data were below the binding occupational exposure level (BOELV, 0.44 mg/m^3 corresponding 2.2 mg/l as urinary total o-toluidine) set under the EU Carcinogens and Mutagens Directive. However, results should be considered carefully due to uncertainties related especially to the limited number of HBM data. There is clearly a need for further HBM data. In addition, further data on the toxicokinetics of o-toluidine in occupational settings focusing especially to the correlations between external intake and urinary levels in occupational exposure would strengthen the assessment. However, if o-toluidine will become authorized under REACH, HBM is recommended to be used to support exposure assessment, since - regardless of the uncertainties – it is only method to inform on the total internal exposure via all routes of exposure (inhalation, dermal, hands-to-mouth).

6.2 Cadmium risk assessment

The RA on cadmium (Cd; **Annex B**) focussed on the older population (>50y), as existing dietary intake limit values were derived based on limit values of proteinuria and urinary Cd levels (U-Cd) for this age category (EFSA, 2009; JECFA, 2010; ATSDR, 2012). The Tolerable Daily Intake (TDI) value of EFSA was based on a critical U-Cd concentration of 1 µg/g crea. The HBM-GV was set at 1 µg Cd/g crea, similar to the value of EFSA and the German HBM-I value. Exposure data for the elderly were available in the EU from two individual studies: France (ENNS) and Spain (BIOAMBIENT_ES). Both show that for the higher exposure percentiles (> P90) the RCR is equal to or above 1. This result should attract the attention of policy makers and emphasize the need to reduce the Cd exposure of the general population. It also confirms the findings by EFSA that based

on external exposure data the margin between the highest exposure categories and dietary limit values is small or even non-existing.

A second approach involved the use of a PBPK model (ANSES, in prep.) for lifetime exposure and prediction of HBM limit values (or 'alert values') at younger age, based on the critical value of U-Cd at age 55-60 years. The European DEMOCOPHES study, which gives an insight of the U-Cd levels for women aged under 45 years in 17 different EU countries could thus be used. It was shown that women aged 35-45 years from Spain, Ireland, Poland and Romania exceeded the age specific U-Cd limit values with more than 5%, meaning that the P95 of exposure divided by the limit values (or the RCR) is >1.

In a next step, the attributable burden of disease (ABD) related to Cd exposure was calculated in women aged above 50 years for chronic kidney disease (CKD) stages 3-5 (exposure-response relationship identified from Akesson et al., 2005; U-Cd threshold set at 1 µg Cd/g crea) and osteoporosis at hip or spine (exposure-response relationship identified from Engström et al., 2011; U-Cd threshold set at 0.5 µg Cd/g crea). The U-Cd threshold for bone effects at 0.5 µg Cd/g crea can be debated, because the causal relationship between low levels of U-Cd and decreased bone mineral density is uncertain, but this uncertainty is taken up in the discussion on the overall uncertainty assessment. The calculation showed that the contribution of Cd to CKD was limited in the ENNS and BIOAMBIENT_ES studies (ABD <1%), while for osteoporosis estimations of the ABD was between 5-10%. Based on the approach in which U-Cd limit values were calculated at younger age and using the DEMOCOPHES data, attributable fraction for osteoporosis varied between 1% (Sweden) and 18% (Poland).

Finally, we stress out that these ADB estimations are preliminary and should not be used by policymakers yet. More data on the risk of osteoporosis at low-dose Cd exposure are necessary. The RA however shows consistently based on external and HBM data that a flag should be raised, indicating the exceedance of the external limit values and HBM guidance values for the higher percentile of exposure.

6.3 Chromium(VI) risk assessment

The work on hexavalent chromium [Cr(VI); **Annex C**] focused on studying whether inclusion of HBM data would improve an existing occupational RA by the Health Council of the Netherlands (2016) on historic exposure to Cr(VI) related to maintenance of military equipment between 1984 and 2006. In addition, REACH authorisation applications for Cr(VI) compounds in surface treatment activities were surveyed regarding their exposure assessment and compared to the available HBM data from Finland covering these activities.

The Dutch RA included a total of 3000 workers that had carried out maintenance activities of NATO equipment with a Cr(VI) primer coating to protect the equipment. For the original RA, exposure information had been very scarce and only crude estimates had been available based on a literature search and extensive job interviews, resulting in a semi-quantitative RA. For the dose-response assessment, a published equation for lung cancer risk was used (Seidler *et al.*, 2013), based on which also the Scientific Committee on Occupational Exposure Limits (SCOEL) and Risk Assessment Committee (RAC) have performed RAs of Cr(VI). These all resulted in very similar cancer risk estimates.

In the current RA, HBM data on Cr(VI) from the Finnish Institute of Occupational Health (FIOH) was used for the exposure assessment. The FIOH database consists of all the HBM samples sent to the Institute for monitoring chemical exposure by the occupational health care units of the work places since 1980s.The samples for which it was possible to identify the worker's job title and/or workplace, were classified according to the Standard Industrial Classification TOL 2008. The HBM

data (p95 values) were converted into corresponding air levels using two previously published conversion equations, and estimated lung cancer risks calculated in the same manner as in the original Dutch RA.

The main conclusion of the current RA was that even though the approach includes several uncertainties, the inclusion of HBM data supported the original Dutch RA and improved its reliability. However, the analysis, focusing on years 1980-1999 did not reflect current exposure levels.

In the reviewed authorisation applications under REACH, Cr(VI) exposure assessment was based, in most of the cases, on modelling even when measured data was available. Furthermore, HBM data was generally not available, and in most of the cases the applicants claimed confidentiality reasons for not presenting the data, even when the companies had biomonitoring programs in place. When compared to the FIOH biomonitoring data from Cr(VI) plating activities, FIOH data showed slightly higher exposure than the estimated reasonable worst case exposure level of 2 $\mu g/m^3$ used in many chromate authorization applications. However, part of this exposure is likely to be derived from exposure via skin, mainly through hands-to-mouth contamination. This does not result in lung cancer but may increase the risk of cancer in gastro-intestinal tract, which is, however, likely to be lower than the risk of lung cancer. In addition, these data, which are based on FIOH measurement data from 2010-2016 cannot inform us on the possible positive impact of REACH authorization.

Current exposure scenarios, a larger variety of jobs and a broader geographical scale should be included, and such studies are being performed within the scope of HBM4EU (under WP 8). Based on the new data, the RA can be updated to reflect the current situation, by applying the methodology presented here. The new study will also generate data that will help us to identify the impact of different exposure routes to total Cr(VI) exposure and will help in replying the policy questions on Cr(VI), which cannot be answered based on the data currently available.

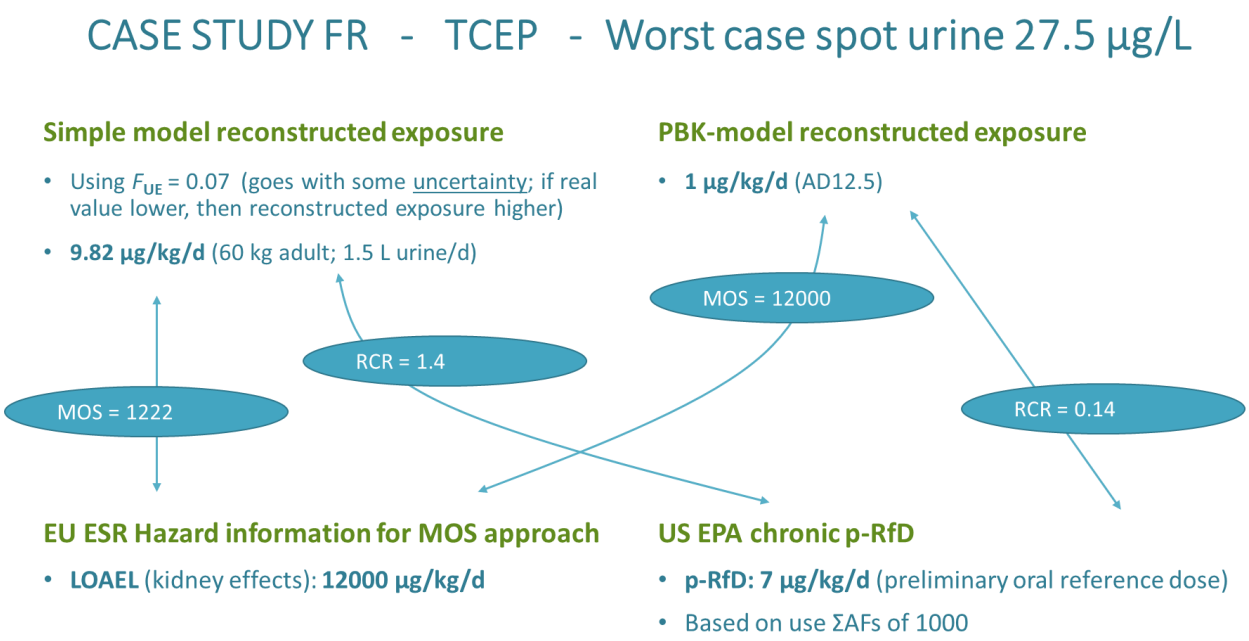
6.4 Flame retardants risk assessment

The work on flame retardants (FR; **Annex D**) initially focussed on developing a system to systematically search and capture relevant data on the chemically diverse 20 FR that were highlighted in the scoping document for “research activities to be undertaken”, based on “evidence of toxicity but a lack of HBM data”. To be able to perform HBM-based risk assessment, the availability of HBM data is a prerequisite as well as regulatory concern and regulatory needs. Regulatory concern has various levels, starting with being earmarked as a Substance of Very High Concern (SVHC) under REACH. Therefore, an overview table was developed to capture information on five main domains of information (with between brackets the working titles used for these domains if not the same): Regulatory need (“Scoping Document”), Regulatory concern (“Regulation”), Exposure, Risk and Hazard. Subsequently, a preliminary (grey) literature search was performed using various sources and based on existing knowledge and experience amongst the authors of this chapter on FR. These were a.o. the Scoping Document (December 2017), websites of ECHA (also searching for pre-REACH assessments), EFSA and US EPA as well as MedLine. The information retrieved was stored in the overview table developed (Annex D10). From the results in the table, it emerged that **TCEP** was probably the most urgent FR to start further searches and work on. It was the only SVHC found in between the 20 highlighted FR as being an SVHC (toxic for reproduction) for which REACH Restriction is being considered.

Subsequently, a case study HBM-based risk assessment was performed for TCEP and was aimed at the general population. Our preliminary search revealed only one paper containing European HBM data (i.e. non-occupationally exposed adults, i.e. general population). This paper by Schindler

et al. (2009) as also referenced in HBM4EU AD12.5 published a urinary range of TCEP biomarker BCEP (bis-(2-chloroethyl)-phosphate). The levels in spot-urine of 16 females and 14 males aged 11-68 years (median 39.5 years) and all but one non-smokers in southern Germany ranged <LOD (0.1 µg/l) – 27.5 µg/l, the median <LOD (0.1 µg/L).

The results are summarised in the figure below.



As no HBM-GV was found for TCEP, risk assessment using HBM exposure data was performed using *model-reconstructed external exposure* starting with HBM data (modelling done by WP12). To this end, initially the simple one-compartmental modelling approach was used for which one publication on the required TCEP-specific urinary molar fraction excreted (F_{UE}) was found (although further scrutiny revealed some doubts on this value). Second, PBK- (physiologically-based kinetic) modelling predicted external exposure result was taken from HBM4EU AD12.5.

For subsequent risk assessment, two approaches were used: the RCR (risk characterization ratio) approach based on the US EPA chronic p-RfD and the MOS (Margin of Safety) approach based on the relevant LOAEL taken from the pre-REACH Risk Assessment Report (RAR).

In summary, it has been shown that the systematic approach developed can help to obtain insight in the most relevant candidate FR for HBM-based risk assessment and clarity regarding in which relevant domains (exposure, hazard, risk) information is available or missing for specific FR's. Using the worst-case German HBM spot-urine measurement result, the case study HBM-based risk assessments on TCEP – that had emerged from the systematic approach as most relevant candidate – indicate low risk (MOS > 1000) when compared to the LOAEL for kidney effects from EU RAR and possible risk (RCR = 0.14 - 1.4) when US EPA p-RfD is used for comparison depending on which exposure reconstruction model is used. US EPA p-RfD was based on BMDL of 6.9 mg/kg/day and used a 1000-fold assessment factor whereas EU RAR used a LOAEL of 12 mg/kg/day as a starting point. This explains why the use of RfD resulted in a possible risk (RCR>1) whereas EU RAR approach didn't (MOS>1000) in the case when same HBM based reconstructed exposure level was used as exposure estimate. The median HBM spot-urine BCEP concentrations were at least a factor of 275 lower compared to the worst-case (27.5 µg/L) used in the RA.

The different outcomes of the two exposure reconstruction models would need further reflection as to the model assumptions, the parameter values including age-dependency: value of F_{UE} in the

simple model, validity children versus adults with respect to volume or urine production, expected half-life and time of sampling in relation to expected highest exposure. Exposure reconstruction using one-compartment model-based approach is a simple model and parameters (F_{UE}) should be selected to give a conservative estimate of external exposure. This estimate can be then refined, when needed, by using measured data and PBPK modelling which can then result in lower estimates.

6.5 PAHs risk assessment

The work on Polycyclic aromatic hydrocarbons (PAHs; **Annex E**) involved a literature review that was performed to find HBM data presenting PAHs exposure in general population and in workers, including the RA calculations. An extensive review of existing literature was made, concerning general population, air pollution, food safety and occupational settings. In addition, calculations of risk based on the data available were made and the following text is divided into two parts: the first part is considering the general population RA, based on inhalation and oral exposure, and the second part is considering occupational RA.

General population – inhalation exposure:

There is no unequivocal biomarker of exposure to atmospheric PAHs in environmental exposure scenarios relevant to the general population and scarce data for some biomarkers such as 3-OH-BaP is found in literature. For that reason, an assumption was made that 1-OH-PYR is an indirect biomarker of exposure to PAH mixtures that include benzo[*a*]pyrene (BaP).

Regarding the literature review, 9 papers were selected that reported 1-OH-PYR HBM data and 3 of them, which also reported air monitoring levels of BaP (external exposure). The latest were used to estimate the excess life-time lung cancer risk following the ECHA-RAC (RAC-Committee for Risk Assessment, 2018) (for detailed calculations see Annex E: PAHs full RA report). The low levels of PAHs metabolites described in urine in these studies, including in FLEHS study, did not allow the back-calculation of exposure levels using HBM in the RAC 2018 approach, thus no values of HBM-based ELCR concerning general population have been determined.

The estimated excess lifetime lung cancer risk, ELCR, at the airborne BaP concentrations reported in above mentioned papers, varied between 6.30×10^{-8} and 3.86×10^{-6}. These were estimated as low, with the exception of one study in France (ELCR=3.56×10^{-5}). As a reference, for a qualitative appreciation of the values obtained, World Health Organisation (WHO) guidance suggests that the unit risk of lung cancer is 87 x 10^{-6} per ng BaP/m^3 for lifetime exposure (WHO, 2000), therefore under the most regulatory programs, an ELCR of 10^{-6} or less represents virtual safety while ELCR greater than 10^{-4} represents high risk (Ambient air pollution by PAHS, (European Commission, 2001).

General population – oral exposure:

The RA for general population due to dietary exposure to PAHs, based of the HBM data was not possible to estimate because no guidance values was found to be reliable for this purpose. Consequently, the ECHA-RAC (2018) dose-response relationships for oral exposure were used to estimate excess lifetime cancer risk[2], ELCR, of dietary (oral) exposure to four PAHs congeners (PAH4: BaA, BbF, BaP and CHR) and to eight PAHs congeners (PAH8: PAH4 + BkF, BghiP, DBahA and IP) (for detailed calculations see Annex E: PAHs full RA report).

[2] Tumours of the liver, lung, forestomach, small intestine, hemangiosarcomas, histiocytic sarcomas and sarcomas of the mesentery, forestomach, skin and kidney (EFSA, 2008)

So calculated cancer ELCRs at the determined exposure doses of PAH4 for mean and high level consumers amounted to $4.02x10^{-5}$ and to $7.11x10^{-5}$, respectively, and the ELCRs at the determined exposure doses of PAH8 for mean and high level consumers amounted to $2.79x10^{-5}$ and to $4.93x10^{-5}$, respectively. These estimates indicate that cancer risk for general mean and high consumers is around 10^{-5}, which means they might not be tolerable. As a reference, for a qualitative appreciation of the values obtained, the indicative tolerable risk level for the general population was considered, that 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).

Alternatively, an attempt has been made to estimate ELCR based on pyrene intake following the ECHA-RAC equations. The pyrene intake was modelled in the HBM4EU WP12.5 (Sarigiannis & Karakitsios, 2018) from the HBM data on the pyrene metabolite 1-OH-PYR. By assuming that pyrene is an indirect marker of exposure to PAH mixtures and that 1-OH-PYR has been linked to dietary exposures (Nethery et al., 2012), the ELCRs were estimated at $9.27\ x10^{-5}$ (non-smokers) and at $4.45\ x10^{-4}$ (smokers). In this approach, the substantial uncertainty related to the proportion of the pyrene compared to the PAH4 and PAH8 mixtures should be taken into account, so these estimates are only approximate and informative (for details see Annex E: PAHs full RA report).

Occupational exposure:

Based on the 1-OHP values the excess lifetime cancer risk (ELCR) for workers, concerning lung cancer, was estimated following the ECHA recent approach (https://echa.europa.eu/fi/applying-for-authorisation/evaluating-applications). ELCR values were calculated using air and HBM data. The approach described for inhalation exposure, based on RAC, was followed. Based on the criteria described, only 7 papers were considered for ELCR estimation. Overall, high ELCR values were estimated (several values higher than 10^{-4}). Moreover, for some studies (3 out of 7) the ELCR estimation using HBM data yielded values higher than those estimated from air monitoring data.

In conclusion, in the presented ELCR estimates, based on the EFSA or WP12 input data, the calculations yielded risk levels of the same order of magnitude (10^{-5}) with an exception for smokers (10^{-4}). As the risk level around 10 times lower (10^{-6}) was estimated using air monitoring data (external measurements), this indicates that using solely air monitoring data may underestimate the risk. Firstly, this might be due to the fact that dietary exposure is the main contributor to the burden of PAH metabolites. Secondly, this may indicate that using external exposure may be suffucient for in risk calculations and that HBM data are crucial for RA. Alternatively, one may consider that using HBM data may overestimate the risk as it does not discriminate between exposure pathways that may important for the cancer related endpoints from PAHs.

Overall, it can be concluded that general use of 1-OH-PYR as a biomarker for PYR exposure and a surrogate of other PAHs exposure, including BaP, generates some uncertainties in risk calculation.

The available approaches for assessing real-life-time exposure to PAHs, and the consequent risks to human population, are not satisfactory and further studies are necessary regarding specific biomarkers for PAHs exposure, or methodologies more sensitive to detect small concentrations of the biomarkers already in use, should be developed to guarantee a more accurate exposure and RA. In addition, considering the review of the literature, it becomes clear that, for attaining a geographical coverage, further HBM data is necessary, from more countries/regions in Europe.

Concerning occupational settings, this work claims attention for two main aspects, namely: i) the exposure levels are still high in some occupational settings and ii) there is a need for developing new occupational studies, applying a set of exposure biomarkers, including a specific biomarker for BaP exposure, which would allow a better ELCR estimation for exposed workers.

6.6 PFAS risk assessment

The work on PFAS (**Annex F**) was built on a recent scientific opinion by the EFSA Contaminants Panel, in which HBM data was used for the exposure and risk assessment of perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) (EFSA 2018), and was of great value. Further, other recent RAs for PFAS were presented and taken into consideration as well. For the work on PFAS under task 5.3, the focus was on mixture RA of PFAS. A preliminary mixture RA of four PFAS was performed, and included the four major PFAS in human tissues: PFOS, PFOA, PFNA and PFHxS.

Great uncertainties were identified in performing the mixture RA, a major one due to species differences with respect to toxicokinetics as well as toxicodynamics, which are specifically relevant for PFAS due to their unique properties. If the mixture RA was based on animal data only, the hazard index was usually below 1, indicating no potential risk. However, if the mixture RA was based on HBM data (from various regions of Europe), human-based hazard data for PFOS and PFOA (cholesterol increases) and extrapolated hazard data using animal-based relative potency factors for PFNA and PFHxS, a hazard index above 1 was usually found, indicating a potential human risk to these compounds.

Three main conclusions were drawn from this work. First, the need for human-relevant hazard and HBM data was highlighted. For the majority of the 4,000 currently used PFAS there are considerable data gaps concerning current uses, exposure patterns and toxicity. Especially, human-relevant exposure and hazard data for PFAS apart from PFOA and PFOS, is needed. Second, endpoint specific relative potency factors based on internal doses in humans are needed. Finally, more intensive collaboration between toxicologists and epidemiologists is called for in order to raise RAs to a higher level.

Further, several issues turned up, e.g how to handle very persistent substances within risk assessment , the need for new methods and approaches for the grouping of chemicals and prediction of their toxicity as well as validation of these methods.

6.7 Phthalates risk assessment

For phthalates, separate RAs were carried out regarding the general population (part A) and the occupational population (part B; **Annex G**).

General population:

The work concerning the general population consists of two parts. The first one discusses the four phthalates (DEHP, BBP, DBP and DIBP) subject to restriction under the REACH regulation (1907/2006). The hazard and exposure assessments as established in the original restriction proposal and the RAC and SEAC opinions are summarised, as well as the risk characterization and uncertainty analysis as discussed in the RAC and SEAC opinion. The focus is on describing the use of HBM data in the restriction, and its strengths and limitations. The second part explores improving the RA by making use of new HBM data. It discusses the impact of the restriction and the role that HBM data could have in quantifying the effects, taking into consideration also information on the substitute phthalates DEP, DMP, DiNP, DiDP and DPHP, and the alternative plasticizer DINCH. RCRs are calculated for DINCH and DEHP using the GVs derived in task 5.2 and HBM data. Finally, employing HBM data to monitor the implementation and effectiveness of the REACH restriction, and for studying time trends of the four restricted phthalates as well as the substitute phthalates are discussed.

The main conclusions were that HBM data were successfully used in the REACH restriction dossier as a reference to determine whether modelled exposure estimates provide a realistic representation of the actual internal exposure. In addition, the HBM data were found to provide an overall estimation of exposure, even though they could not be used to determine the contribution of different sources. Regarding RCR calculation based on HBM data, various factors were found to induce uncertainty. It was additionally illustrated that the RCRs depend on the methodology used to express hazard and exposure (i.e. internal concentrations or urinary metabolite concentrations).

Furthermore, it was concluded that there is potential for HBM to assess the impact of the whole EU legislation regarding phthalates retrospectively. The data available showed an overall declining trend in the use of phthalates with the exemption of DiNP, while the alternative phthalates DiDP and DPHP, and the alternative plasticiser DINCH showed an increasing trend. To observe whether the restriction has a positive impact in the effective reduction of the exposure to phthalates, data from 2017 and onwards must be generated, preferably on an EU wide scale. HBM4EU therefore may play a valuable role in monitoring the effectiveness of chemicals legislation, including the phthalates restriction.

Occupational population:

The work concerning the occupational population covered DiNP, DiDP and DPHP, because their use has not been restricted and they are widely used in plastic product manufacturing. Earlier occupational RAs for these three phthalates could not be identified. Furthermore, occupational exposure limit (OEL) values have not been derived for DiNP, DiDP or DPHP, neither for internal nor external exposure. For DPHP however, a provisional EU HBM-GV will be available for the occupational population, currently under revision in task 5.2 and employed in this assessment as indicative. For the RA of DiNP and DiDP, the BE methodology was applied, based on DNELs previously derived by ECHA. Published HBM data was used for the exposure assessment, and RCRs calculated.

The main conclusion was that even though the BE approach is a rough method, in many cases it can be considered sufficient for an approximate RA, giving a first estimate of risk levels. For instance, here, the calculated RCRs were well below one for DiNP and DiDP, although conservative assumptions were made. However, there were some uncertainties related to this approach, which must be recognized. These included the short half lives of the phthalatates and the use of single spot sampling for the measurement of biomarker levels. The equation used for the BE derivation assumes steady state urinary levels, which is not likely to be true in the case of occupational exposure to short half life substances. However, when the sample is taken as a post-shift sample, it often represents a peak level and results in an overestimation of exposure rather than underestimation. This can be confirmed if several samples at different time points are taken to elucidate the kinetics of the substance after the exposure. This type of RA would also benefit from further elaboration by PBPK modelling. Moreover, in this case, only individual phthalates were assessed, but it is known that mixtures of phthalates may have direct additive effects.

Importantly, the current data on occupational exposure to these three phthalates is very limited, and therefore also the exposure assessment should be viewed with caution. As these phthalates are being used to replace the already restricted phthalates, more occupational exposure data on them is needed. As the use of these newer phthalates will likely increase and the exposure levels will subsequently rise in the general population, distinguishing low occupational exposures will become more difficult.

On a more general level, HBM appears important for the exposure assessment of phthalates, as they are not particularly volatile. Their air concentration measurements can therefore be

misleading, and exposure via inhalation may not be the only significant route. Thus, also the other exposure routes should be considered in the exposure assessment, making biomonitoring an important tool.

7 Discussion and general conclusions

7.1 Use of HBM in the risk assessment of 1st priority group compounds

RA for chemicals can be reliably performed only when there are enough data available to support it. However, for many compounds, lack of data is reality, and yet they are being used and different populations are exposed to them. During the recent years, modelling of exposure has become more and more important in regulatory RA. This applies especially to industrial chemicals under the REACH regulation, substances in biocidal products and plant protection products. However, modelling includes a lot of uncertainties, although they can be reduced by the use of measurement data to support the modelling. This often means external exposure monitoring data, i.e. air monitoring data or measured data on contaminant levels in the environment or food, which are used to support RA. However, HBM could provide more accurate data on the total internal exposure, covering all routes of exposure.

The main challenge in the use of HBM data has been difficulties in its interpretation and comparing it to health-based limit values, which are typically set for external exposure. When it has not been clear, how e.g. urinary metabolite levels can be compared to DNEL/TDI/etc. values, given for external exposure, biomonitoring data have often been ignored. For this reason, Hayes and co-workers (Hays et al., 2009) developed the BE concept, which has been applied for example in the setting of German HBM health-based guidance values (HBGVs), and now in the setting of HBM4EU GVs. In its simplest form, the BE approach involves the use of a simple one-compartment-model based formula to calculate urinary substance or metabolite levels corresponding to external DNEL/TDI/etc. values.

As exemplified in the occupational RAs for phthalates and o-toluidine, a rough RA using HBM data is often possible using this approach. The only substance-specific information needed is the fraction of the compound or its metabolites excreted into the urine during 24 or 48 hours. When there are uncertainties related to the excretion kinetics, a conservative estimate on the excreted fraction can be used in the first RA. In the case of occupational RA of phthalates and o-toluidine, post-shift urinary levels were used for BE calculations. Since in the case of short-lived substances, post-shift levels often represent peak levels rather than steady-state levels, additional conservativeness is brought to the assessment. This approach can be seen and employed easily for RA screening purposes (1st tier assessment using HBM) to get an overall view on whether a concern for adverse health effects may exist. It can also be used when there is a need for supporting data to complement external measurement and modelled data. In many cases, this approach is sufficient to show that risks are unlikely at the measured exposure levels. However, if RCRs close to 1 are observed, more refined assessment might be needed to reduce uncertainties (2nd and 3rd tier RA based on HBM data).

In more advanced RA, uncertainties related to the use of HBM data are reduced by measured and modelled toxicokinetic data in humans. Measured data may include, for instance, controlled exposure studies, using oral or inhalation exposure at levels relevant to environmental or occupational exposure, depending on the exposure scenarios. Measured data on the correlations between air levels and urinary levels are available also from occupational settings. This kind of data was used for example in the Cr(VI) RA. However, the measured correlation data came from Cr(VI) plating and was not very recent, which created uncertainties when extrapolating data to other tasks and current exposure scenarios.

In the case of cumulative, long half-life substances, a RA based on HBM is absolutely superior when compared to a RA based on external exposure assessment. In many such cases, it would be difficult to perform a reliable RA based on external exposure. In the case of cadmium and PFAS, it

has been possible to derive dose-responses directly for biomarker levels based on human epidemiological data. Thus, measured levels in different populations can be directly compared to these levels. On the basis of the Cd assessment performed here, both external and HBM data consistently indicates the exceedance of the external limit values and HBM guidance values for the higher percentile of exposure.

The RA of PFAS highlights the importance of toxicokinetics, due to the significant differences in the half-lives of PFOA/PFOS between humans and rodents. The current provisional opinion of EFSA is therefore based on human data and shows exceedances of the TWI of a considerable part of the European population. Critical endpoints identified were effects on lipid metabolism, immune toxicity and reduction of birth weight. Here, the approach was to move a step forward from the existing RAs, and assessment of PFAS mixtures was attempted. When the mixture RA was based on HBM data, human-based hazard data for PFOS and PFOA (cholesterol increases) and extrapolated hazard data using animal-based relative potency factors for PFNA and PFHxS, a hazard index above 1 was usually found, indicating a potential human risk to these compounds, which is in line with the provisional EFSA opinion. However, the assessment includes several uncertainties, and e.g. more hazard data on humans and potency factors based on internal doses in humans are needed to refine the assessment.

RA of complex mixtures based on HBM data is a challenge, which is exemplified also by the PAHs RA. Usually 1-OH-PYR, the main metabolite of pyrene, is used as a biomarker for pyrene exposure and a surrogate for other higher molecular weight PAHs, including BaP. However, pyrene itself is not carcinogenic and monitoring of this metabolite for the purpose of RA is only based on the assumption that its level correlates with BaP and other carcinogenic species co-occurring commonly with PYR in PAHs mixtures. This generates additional uncertainty in the RA, since the proportion of metabolites varies in different PAH mixtures. In addition, exposure to PAHs mixture may modify metabolism or induce different effects, therefore a single metabolite may not adequately characterize exposure to PAHs. Even though there are methods available for biomonitoring of 1-OH-BaP, and established correlations between 1-OH-BaP levels and external exposure, the method hasn't been used often, since high sensitivity is needed to detect low BaP metabolite levels. The RA could be refined using the data on 1-OH-BaP levels. Exposure biomarker data on other PAHs could be also used to refine RA by using the toxic equivalency factor method for mixture RA.

Overall, even though RA based on HBM data includes uncertainties, it is important to note that this is always more or less the case with RA. Importantly, the uncertainties related to the use of HBM are often smaller than those related to exposure assessment based on only environmental concentrations and/or modelling. In the best case, HBM and external exposure assessment and modelling can support each other, giving high confidence to the exposure assessment. Naturally, the quality of the HBM data must be high, and the risk assessor needs to have expertise to interpret it. Work on generating guidance on the aspects to consider when using HBM data in RA is planned within task 5.3.

7.2 Results of the RAs in the light of policy questions

In the case of **anilines**, RA of o-toluidine was made, which suggests a cancer risk of 1:20 000 in the worst-case scenario. However, it should be noted that the exposure data used for the assessment was very limited, and therefore the RA should be seen as an example. However, an approach how HBM data can be used to support the RA of o-toluidine was introduced. This may be important if o-toluidine will become authorized in future.

For **cadmium,** a risk assessment, as well as an estimation of Cd exposure ABD, was implemented taking into consideration HBM data.

Evidence of significant exceedance of the U-Cd threshold value of 1 µg/g crea for women at age around 60 years is observed from a Spanish and French HBM studies and also for most of 14 EU countries having participated in the DEMOCOPHES HBM study (results based on PBPK modelling and dietary intake assumptions).

The obtained results lend further support to the EFSA conclusion that Cd exposure in the general population should be reduced. It even strengthens this recommendation, as when considering the aggregated exposure to Cd through available HBM results, the fraction of women exceeding the U-Cd threshold value for renal effects could reach up to 32% (DEMOCOPHES - Poland results).

However, as the selected HBM datasets (BIOAMBIENT_ES, ENNS, DEMOCOPHES) came from a non-smoking, ex-smoking or smoking female population, further scrutiny of this important exposure determinant could help discriminate between the contribution of environmental tobacco smoke versus the contribution of dietary intake for the Cd highly exposed individuals.

The **hexavalent chromium** RA was focused on the refinement of a Dutch RA on the military use of Cr(VI) before the year 2000, by using Finnish biomonitoring data from 1980-1999. In addition, Finnish data on occupational exposure in Cr(VI) plating activities from 2010-2016 was compared to exposure estimates made under REACH authorizations. Since the data was limited to Finland and pre-authorization period, the policy questions on chromium can be better answered when new, EU-wide data on occupational exposure to chromium appears from the Chromate study performed under WP8. The study involves also the development of new, more specific biomarkers for Cr(VI) biomonitoring, which could possibly inform also on general population exposure to Cr(VI), which cannot to be assessed on the basis of measurement of urinary Cr, which is the commonly used biomarker for Cr.

For **FR**, two policy questions considered risk: (11) “Can exposure to FRs be linked with any adverse health effects?” and (14) “What additional FRs should be prioritized for further information regarding exposure and/or toxicity? How can use and risk information be combined to identify and prioritize knowledge gaps for further assessment?”. For question 14, a systematic approach including a structured table in which results should be further filled and regularly updated was prepared to capture up to date information on regulatory status, regulatory needs, exposure (external and internal), hazard (epidemiological and in vitro, in vitro and in silico toxicological information) and analytical capacity (suitable biomarker). The preliminary filling of the table revealed that TCEP would be a relevant FR for which a regulatory need exists for further HBM data (REACH Restriction under preparation considered).

Regarding question 11, only few results could be obtained. The number of European HBM data sets on TCEP was very limited (one adult data set, one on children aged 22 months – 80 months, both in Germany). Furthermore, no REACH DNEL or EFSA TDI was found, nor a WHO TDI, only a US EPA p-RfD (preliminary oral chronic reference dose) and a LOAEL was found that was used as a basis for risk assessment in the pre-REACH era (both based on kidney effects). Albeit with some uncertainty as to the kinetic modelling (results) used, preliminary risk calculations indicate possible risk for adverse health effects (kidney effects) for the worst-case exposed adult in the German study. This policy question will probably be easier to answer when new data will become available for FR in the aligned studies. This is expected for the end of 2019. Furthermore, research to establish substance-specific proportional urinary excretion fractions (an important parameter for first tier (one-compartment) kinetic modelling) for FR, may help to interpret new HBM data on more FR in a risk context as long as substance-specific HBM-GV are not available.

For **PFAS**, the current exposure seems to exceed the guidance values in some parts of the European population. The human exposure levels and human hazards to PFOS and PFOA are relatively well established. The health effects are mostly related to disturbed lipid metabolism, reduced birth weight and impaired immune responses according to the current state of the science. However, for the majority of the remaining PFAS, large data gaps exist. Preliminary mixture risk assessments can be estimated based on animal hazard data and HBM data. However, this method may underestimate the risk due to great species differences. The new data being generated within HBM4EU should help to further answer the policy questions related to PFAS.

A major advantage of HBM data is that it provides an integrated overview of the body burden selected chemicals and serves as a good approximation of aggregate exposure. This is of particular relevance for **PAHs**, since besides ingestion and inhalation routes, dermal absorption can also have an important role particularly in occupational exposures.

However, environmental/HBM studies in the EU should be standardized allowing a more accurate comparison of the PAHs levels across all Europe and standardized publication of the data. Moreover, the available approaches for assessing real-life exposure to PAHs, and the consequent risks to human population, are not satisfactory and further studies are necessary regarding specific biomarkers for BaP exposure, or methodologies more sensitive to detect small concentrations of the biomarkers already in use, should be developed to guarantee a more accurate exposure and risk assessment. In addition, considering the review of the literature, it becomes clear that, for attaining a geographical coverage, further HBM data is necessary, from more countries/regions in Europe.

Overall, it can be concluded that general use of 1-OH-PYR as a biomarker for PYR exposure and a surrogate of other PAHs exposure, including BaP, generates some additional uncertainties in risk calculation.

Concerning occupational exposures, there two main aspects to consider: i) the exposure levels are still high in some occupational settings and ii) there is a need for developing new occupational studies, applying a set of exposure biomarkers or a specific biomarker for BaP exposure, which would allow a better ELCR estimation for exposed workers.

For **phthalates**, the RA of DEHP, BBP, DBP and DIBP that is part of the restriction dossier of 2017 was used as the starting point of this assessment. In this RA, DEMOCOPHES HBM data was already integrated. We conclude that HBM data provides a valuable means to validate the exposure modelling usually performed in a RA. Furthermore, we conclude that it is difficult to obtain data that is representative for the entire population, including sensitive subgroups, and harmonisation of data is often a challenge due to different use of methodology. HBM data allowed us to observe time-trends with regard to the impact of the restriction under REACH and to observe a potential shift towards substitute phthalates or the alternative plasticiser DINCH. However, more data representing the timeframe after the restriction was put into place (post-2017) will strengthen this analysis. The data generated under WP8 will lead to more harmonized, recent data to further explore these policy questions. Mixture assessment covering more phthalates than the four subject to the current RA will be another topic of attention in the further course of the project. Concerning the occupational exposure to DiNP, DiDP and DPHP, the HBM data is very limited. The current health risk of these three phthalates appear to be low based on the available data, but the RA should be updated when more data are available.

8 Proposals and recommendations for the better inclusion of HBM in risk assessment and health impact assessment

Better inclusion of HBM in regulatory RA and HIA would be very beneficial in many cases to help improve their reliability and to reduce the uncertainties associated with RA/HIA. However, the use of HBM itself is often thought to include uncertainties, hindering its inclusion. Nevertheless, as shown here, there are several approaches that can be taken also when there are no existing biological limit or guidance values. In such cases, a tiered approach for the inclusion of HBM is recommended, starting from a simple 1st tier approach and requiring increasing amounts of information for the higher tier methods.

The recommended 1st tier method is reverse/forward calculation based on simple one-compartment model to relate existing external guidance values and measured internal doses (Hays *et al.*, 2008). This approach is simple and rough, and uses only very basic parameters. Therefore, it may be used as a screening method for placing HBM data into a health risk context. However, in many cases this approach can be considered sufficient, especially when conservative assumptions have been used for the F_{UE}, and the calculated RCRs remain well below 1, indicating a low risk. Nevertheless, in cases where the RCR is closer to 1, a more detailed approach may be needed to refine the risk assessment. For the 2nd tier, and when more data is available on toxicokinetics, PBPK modelling is recommended. For the most robust, 3rd tier approach, measured data on correlations between external exposure and internal doses would be needed, e.g. from controlled volunteer studies or comprehensive studies in occupational settings.

There are certain cases where inclusion of HBM would be particularly important for performing RA. This applies, e.g., for compounds such as PAHs and Cr(VI) for which skin exposure (or hands-to-mount exposure) is significant, since there are significant uncertainties related to dermal exposure modelling. HBM provides data related to the total body burden. Gathering additional contextual data, which should always be done, would also allow identifying exposure sources and routes. Collecting suitable contextual information would also guarantee that HBM will be a useful resource for risk management and regulatory action. Moreover, in cases where exposure can occur through both food consumption and the work environment, HBM can be used to study how much the occupational exposure adds to the total exposure.

Additionally, HBM would be particularly useful for cumulative compounds, such as PFAS or cadmium. In fact, for cumulative compounds, reliable exposure assessment is not even possible using other methods. In addition to exposure assessment, for cumulative compounds also hazard assessment may be based on correlations between HBM levels and health effects, or key events (KEs) in adverse outcome pathways (AOPs). If the NOAELs/BMDs derived in toxicity studies could be linked with biomarker levels and KEs in AOPs, HBGVs could be derived directly and more accurately. This would of course require gathering data on biomarker levels also in toxicity studies, or human data on early effect markers. A well-known example of these are early biomarkers for renal tubule damage, which have been shown to correlate with urinary cadmium levels.

One of the major challenges for the inclusion of HBM into RA, particularly when 2nd or 3rd tier approaches are required, is the often limited data available on toxicokinetics. In these cases, for example PBPK modelling is problematic. Furthermore, information on the differences in toxicokinetics between species would be very important. For example, long chain PFAS accumulate in humans to a much greater extent than in rodents and monkeys, underlining the relevance of human data for their RA.

As exemplified for instance by the PAHs and Cr(VI) RAs, in some cases there is an urgent need for more specific biomarkers or more sensitive analytic methods than currently available. For example,

regarding PAHs, the general use of 1-OH-PYR as a biomarker for PYR exposure and a surrogate for other PAHs exposure, including BaP, generates some additional uncertainties in risk calculation. Further studies are necessary to find more sensitive methods for the analysis of BaP and/or other PAHs biomarkers.

For the substances on the HBM4EU's first list of priority substances, more HBM data is needed in general, as well as data covering more European countries and regions. This work is ongoing in WP8, and the RAs presented here will be updated when new data becomes available.

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Annex A: anilines risk assessment

D5.5 Substance-group specific risk assessment for anilines (ortho-toluidine)

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1 Introduction

Aniline and many of its derivatives are known or suspected human carcinogens. Several aniline derivatives can be found also from the candidate list of substances of very high concern (SVHCs) and the list of substances restricted under REACH. This risk assessment is focused on **ortho-toluidine** (CAS 95-53-4), which is a carcinogen, classified to cat 1B according to CLP. o-Toluidine has been added in year 2012 to the candidate list for eventual inclusion in Annex XIV to REACH. Inclusion in annex XIV to REACH means that in future the use of o-toluidine requires authorization in EU. For authorization purposes users of the o-toluidine need to demonstrate the adequate control of exposure by providing reliable and representative exposure information from their uses. HBM data could help in this if the measured biomarker levels can be used to cancer risk assessment. This RA exercise demonstrates how this can be made.

o-Toluidine is manufactured and/or imported in EU in quantities of 10 000 – 100 000 tonnes/a. The main uses of o-toluidine include: curing agent in epoxy resins and as intermediates in producing azo dyes and pigments, acid-fast dyestuffs, triarylmethane dyes, sulphur dyes, indigo compounds, photographic dyes and synthetic rubber and rubber vulcanising chemicals. The largest use is, however, as an intermediate in the manufacture of herbicides.

o-Toluidine is considered to be an animal and human carcinogen, and may cause methemoglobinemia in humans (SCOEL 2017). Therefore, there is a need for limiting the risks especially for workers although consumers should also be considered. To further understand these risks, it is important to have more knowledge on exposures of both workers and the general population.

The policy-related questions relevant for T5.3 and anilines are:

- What is the current occupational exposure to anilines and MOCA in EU?
- What is the impact of REACH on levels of anilines? (Feed HBM data into risk assessments of anilines and MOCA)

Current information related to MOCA (CAS 101-14-4, 2,2'-dichloro-4,4'-methylenedianiline) is considered sufficient and further research activities related to MOCA are not considered relevant in HBM4EU. MOCA is currently authorized under REACH and estimated number of exposed workers in EU is only about 200.

In this risk assessment document, we aim to summarize toxicity data on o-toluidine, and further investigate available exposure data on o-toluidine in the occupational field. Using this information, we conduct a risk characterization by applying the biomonitoring equivalent (BE)

methodology. In the last section of this document we discuss the results and provide further recommendations.

2 Methodology

For the hazard assessment of o-toluidine we summarized available (dose-response) data from existing risk assessments. A literature search via PubMed was conducted to study available data biomonitoring data. The Biomonitoring equivalent (BE) methodology was used for the comparison to roughly estimate the urinary levels corresponding the external intake levels (or vice versa). For this purpose, the approach by Angererer et al. (2011) was used. For the risk assessment we compared the results of the BE approach to the available biomonitoring studies.

3 Summary of hazard characterisation

In rats and upon oral exposure, o-Toluidine is rapidly absorbed via gastrointestinal tract, rapidly distributed, metabolized and excreted (mainly via urine). In rats, 50 mg/kg o-toluidine administered subcutaneously was extracted to urine 74 % in 24 h and 92 % after oral dosing as metabolites or unchanged. The saturation of metabolic pathways is not as apparent in the urinary excretion data. Absorption via skin as well as respiratory tract routes is shown by data from acute toxicity studies (OECD 2004).

Oral dose of 500 mg toluidine/kg bw to rats a half-life time of plasma elimination of 12-15 hours was derived and i.v. application of 111 mg/kg bw to dogs yielded a half-life time of plasma elimination of half an hour. Metabolism studies show that N-acetylation and hydroxylation of the aromatic ring of o-toluidine are the major metabolic pathways in rats. Sulphate conjugates predominate over glucuronides by a ratio of 6:1. The metabolites which were found in the urine of rats included azoxytoluene, o-nitrosotoluene, N-acetyl-o-toluidine, N-acetyl-o-aminobenzyl alcohol, 4-amino-m-cresol, N-acetyl-4-amino-m-cresol, anthranilic acid, N-acetyl-anthranilic acid, 2-amino-m-cresol and unchanged o-toluidine. Faecal excretion and exhalation of the substance was minimal with up to 3.5 % of the dose (OECD 2004, NTP 2011).

Studies of human o-toluidine metabolism were not identified. However, metabolic pathways are expected to be similar to those reported in experimental animals based on studies of other aromatic amines and knowledge of the key metabolizing enzymes (NTP 2011).

The acute toxicity of o-toluidine is reported to be: LC_{50} (rat) is 852 ppm/4 hrs (approx. 3827 mg/m^3/4 hrs), oral LD_{50} (rat) is 750 mg/kg bw, and dermal LD_{50} (rabbit) is 3250 mg/kg bw. The predominant symptoms after inhalation or oral application were cyanosis, laboured breathing, lethargy or loss of consciousness. o-Toluidine is a methaemoglobin forming chemical which have been demonstrated in rat, cat and humans (OECD 2004, SCOEL 2017).

Methaemoglobin levels elevated up to 19 % in the subacute feeding study as well as in the marked splenic toxicity in the subacute gavage and sub-chronic feeding studies leading to hyper-cellularity in the bone marrow. Other target organs were liver and kidney (hemosiderin deposition) and urinary bladder (hyperplasia). A NOAEL could not be derived, the LOAEL (rat, 14-day feeding study) was 500 ppm (approx. 23.7 and 25.5 mg/kg bw/day for males and females, respectively) (OECD 2004).

o-Toluidine is not a skin-irritant when tested for 24 hours under semi-occlusive conditions (rabbit, ear) and is moderately irritating when tested 24 hours under occlusive conditions (rabbit, skin). o-Toluidine causes serious damage to the eyes of rabbits (OECD 2004).

o-Toluidine has been listed as a human carcinogen (Group 1, IARC 2012) based on sufficient evidence of carcinogenicity from studies in both experimental animals and humans. o-toluidine causes DNA and chromosomal damage and induces mutations and is therefore considered as a carcinogen with a genotoxic mode of action. In humans, occupational exposure to o-toluidine has been mainly associated to the urinary bladder cancer, but the human data is not adequate for risk assessment (SCOEL 2017). Genetic polymorphism for N-acetyltransferases in humans can increase susceptibility to aromatic amine-induced cancer for slow-acetylators (Golka et al. 2002).

The Scientific Committee on Occupational Exposure Limits (SCOEL 2017) estimated the urinary bladder cancer risk of o-toluidine using the results from a two-year rat feeding study (NCI, 1979). Since no adequate epidemiological data was available, the data on the formation of the transitional-cell carcinomas of the urinary bladder in female rats reported in that study was taken as most relevant for hazard characterisation. The rat cancer study was regarded suitable for dose response modelling, and resulted in a Benchmark Dose causing 10 % tumour incidence above background level (BMD_{10}) of 42.2 mg/kg bw per day.

4 Summary of available exposure assessment

A PubMed literature search was conducted on o-toluidine in combination with occupation* and exposure or air. This resulted in two relevant papers with exposure information. Also, a review chapter by National Toxicology Program was retrieved (NTP, 2011).

Korinth et al. (2007) measured the workplace air by personal air monitoring for o-toluidine exposure in the automobile industry. The average exposure in non-smoker workers was 61.4 µg/m^3 and the 95th percentile 524.0 µg/m^3, which corresponds approximately to 75 µg/kg bw/d assuming 10 m^3 inhaled air during working shift for 70 kg worker. Hanley et al. (2012) measured o-toluidine exposure during various processes in a rubber chemical manufacturing plant. They found a GM of 0.07 ppm (approx. 307 µg/m^3) and maximal air concentration was 0.37 ppm (approx. 1.62 mg/m^3).

According to a review by the National Toxicology Program (2011) most of the air-monitoring data in the United States suggest that occupational exposure levels of o-toluidine are usually less than 1 ppm but may have been higher in the past. Note that dermal exposure might also be considerable since o-toluidine can easily be absorbed through the skin (Korinth et al. 2007). In Europe, most people exposed to o-toluidine (about 5500 in total) work in the manufacture of chemicals, chemical products and man-made fibres or manufacture of rubber products (Cherrie et al. 2011).

The current OEL values vary across EU countries from 0.44 to 9 mg/m^3. EU binding occupational exposure level value (BOELV) of 0.1 ppm (0.44 mg/m^3) must be adopted by member states in year 2020. This has been set based on SCOEL dose-response model and taking into account the socio-economic considerations.

The data of the o-toluidine levels in the urine of general population has been used to set a biological guidance value (BGV) for urinary o-toluidine (SCOEL 2017). Based on the studies by Kütting et al. (2009) and Weiss and Angerer (2005) the 95th percentile of urinary o-toluidine (free and conjugated) among the non-smoking general population is approximately 0.2 µg/l. The urinary concentration of 0.2 µg/l has been set as a BGV in Germany for o-

toluidine measured from post-shift urinary samples in the end of the working week. Since smoking increases urinary o-toluidine levels, this value applies only for non-smokers/can be applied only after abstaining from smoking.

5 Risk characterisation

The existing cancer risk assessment conducted by SCOEL (2017) resulted in a Benchmark Dose causing 10 % tumour incidence above background level (BMD_{10}) of 42.2 mg/kg bw per day in rats. The BMD_{10} of 42.2 mg/kg bw per day was calculated to correspond to an inhaled dose of about 840 mg/m^3 as an 8-hour TWA at occupational exposure during 40 out of 75 years, 48 out of 52 weeks and 5 days/week (assuming a body weight of 70 kg, an inhaled amount of 10 m^3 for 8 h working day, and equal absorption via inhalation and the oral route, i.e. 42.2 mg/kg bw per day * 70 kg / 10 m^3 * 75/40 * 52/48 * 7/5). Allometric scaling of this dose level of 840 mg/m^3 from rat to human using a default value of times 0.25 provides a POD for 10 % increase in tumour risk of 210 mg/m^3 (48 ppm).

This resulted in following risk estimations:

A tumor risk of 1 : 10 at the BMD_{10} of 210 mg/m^3 (48 ppm)

A tumor risk of 1 : 1000 at 2.10 mg/m³ (0.48 ppm)

A tumor risk of 1 : 10 000 at 0.210 mg/m^3 (0.048 ppm)

A tumor risk of 1 : 10^6 at 2.10 µg/m^3 (0.00048 ppm)

In order to estimate risk figures for occupational exposure, it has to be assumed that occupational exposure is the only exposure so the same external exposure levels in mg/kg bw/day are recalculated to be anticipated to have occurred during 8 h/day and 5 d working week: Converted to mg/bw/working day (as occupational exposure 8h/day, 5 d/wk, 70 kg worker, inhaling 10 m^3 of air during the working day) these figures are:

A tumor risk of 1 : 10 at the BMD_{10} of 30 mg/kg/day

A tumor risk of 1 : 1000 at 0.3 mg/kg/day

A tumor risk of 1 : 10 000 at 0.03 mg/kg/day

A tumor risk of 1 : 10^5 at 3 µg/kg/day

A tumor risk of 1 : 10^6 at 0.3 µg/kg/day

6 Improving the RA by biomonitoring

6.1 Evaluation of the available biomonitoring data

In general, all studies measured o-toluidine after hydrolysis of the conjugated metabolites from urinary samples.

Labat et al. (2006) measured the urine concentration of ortho-toluidine among individuals who worked in a French liquid SO_2 plant polluted with ortho-toluidine. Pre-shift, the urine concentration of o-toluidine ranged between 1.7 ± 1.5 µg/L while the post-shift levels increased to 523 ± 321.6 µg/L.

Korinth et al. (2007) assessed occupational exposure to aniline and o-toluidine among people (46 men, 5 women 20-61 years) working in the manufacturing of rubber products. The

measured total urinary o-toluidine concentration from post-shift samples was 38.6 µg/L (mean) and 292.4 µg/L (95th percentile).

Li et al. (2011) examined the relationship between DNA-damaging chemicals and average telomere length for 157 workers, working in the Swedish rubber industry. Ortho-toluidine was measured in urine samples acquired during the last four hours of an eight-hour shift, leading to a concentration that ranged between 0.03 and 0.11 µg/L.

Urine excretion of aromatic amines including o-toluidine were investigated by Seidel (2005) in an effort to link them with human bladder cancer. Urine samples were collected in 24 hours for 81 non-smoking individuals (48 women) aged 20-61 years, in Germany. The median and maximum of o-toluidine was found to be 61.8 ng/24h and 401 ng/24h respectively.

Another study associated with carcinogenic aromatic amines was conducted by Riedel et al. (2006) in Germany. A 24-urine sample was collected from 20 people (10 smokers). The non-smoking group had a mean o-toluidine concentration of 167 ± 199.4 ng/24h while the concentration for the smoking group was found to be 204.2 ± 59.1 ng/24h.

Lindner et al. (2011) determined the exposure of smokers and non-smokers to smoke constituents like o-toluidine. The study observed the urine concentration of o-toluidine in 24-hour samples taken in three countries (Germany, Switzerland and United Kingdom). 1631 adult individuals (1223 smokers) took part in the study. Exposure levels were lower in non-smokers than smokers with mean o-toluidine urine concentrations of 64 ± 128 ng/24h and 179 ± 497 ng/24h respectively.

There is some information available on the background levels of o-toluidine haemoglobin adducts within the general (non-occupationally exposed) population. In the study by Weiss and Angerer (2005) the median level of haemoglobin adducts among 200 representatives of the general population was 22.6 ng/l and the 95th percentile was 82 ng/l (range <LOD - 5929 ng/l). The mean levels vary typically between 2 ng/l (0.03 ng/g Hb) and 50 ng/l (330 pg/g Hb). Mean o-toluidine Hb-adduct levels have been 10 times higher in exposed versus unexposed workers and >100 times higher than the means in unexposed populations previously studied (Hanley et al., 2012).

Biomonitoring equivalent (BE) methodology was used for the comparison to roughly estimate the urinary levels corresponding the external intake levels (or vice versa). For this the following formulas are used:

$$Css = \frac{\mathrm{D} \times \mathrm{BW} \times \mathrm{Fue}}{\mathrm{V24}} \quad \text{or} \quad D = \frac{\mathrm{Css} \times \mathrm{V24}}{\mathrm{Fue} \times \mathrm{BW}}$$

Here, D is the external dose as mg/kg bw, C_{ss} is the urinary level of the substance at steady state (mg/L), V_{24} is the estimated average 24-hour urinary volume (L), and F_{UE} is the mass of o-toluidine, including hydrolysed conjugate metabolites, excreted to the urine during the 24-hour per mass of parent compound ingested (percentage) (Angerer *et al.*, 2011).

For German non-smoking rubber workers (Korinth et al. 2007) which showed median and 95th percentile U-o-toluidine levels of 6 and 292 µg/L in post-shift samples a rough estimate of the total dose is 0.17 µg/kg and 8 µg/kg. These urinary levels are assumed to represent a steady-state level and 75% of the dose is assumed to be excreted to the urine as measured parent compound including hydrolysed conjugate metabolites (=F_{UE}) which is based on urine excretion observed in s.c. dosed rats after 24 h (OECD 2004). A value of 1.5 L/day was used as the 24-hour urinary volume (V_{24}) and 70 kg as the average body weight was used in the calculation.

Respectively, using this BE approach:

A tumor risk of 1 : 10 000 at 30 µg/kg/day (0.048 ppm) (corresponds approximately to a steady state urinary level of 1 mg/l calculated by using the BE methodology)

A tumor risk of 1 : 10^5 at 3 µg/kg/day (0.0048 ppm) (corresponds approximately urinary level at steady state of 100 µg/l calculated by using BE methodology)

A tumor risk of 1 : 10^6 at 0.3 µg/kg/day (0.00048 ppm) (corresponds approximately urinary level at steady state of 10 µg/l calculated by using BE methodology)

A calculated BE corresponding to the new BOELV of 0.1 ppm (0.44 mg/m^3 = 0.06 mg/kg) is 2.2 mg/l.

6.2 Risk assessment for the sample populations

In German rubber industry workers urinary o-toluidine values (95th percentile level) of 292 µg/L have been measured. According to the calculations presented above, this corresponds the cancer risk level of 1 : 37 000.

In a French liquid SO_2 plant polluted with o-toluidine (Labat et al. 2006) post-shift urine levels up to 523 ± 321.6 µg/L were measured in workers. This corresponds to approximate tumor risk level up to 1 : 20 000.

The 95th percentile of urinary o-toluidine (total of free and conjugated) among the non-smoking general population is approximately 0.2 µg/l (Kütting et al. 2009; Weiss and Angerer 2005). This 95th percent urinary concentration of 0.2 µg/l has been set as a BGV (biological guidance value) in Germany for o-toluidine measured from post-shift urinary samples in the end of the working week. This o-toluidine concentration indicates a tumor risk lower than 1 : 10^6. Smoking can increase o-toluidine levels 2-5 times in general population.

7 Discussion and conclusions

The Bioequivavalent (BE) methodology using an one-compartment model based formula (Angerer et al. 2011) was utilized in this o-toluidine assessment. This BE method can calculate mainly steady-state levels; this must be considered with rapidly eliminating chemicals because occupational exposure levels can vary during working day. The half-life of o-toluidine is approximately four hours and the majority of available HBM data is based on pre- and post-shift urinary spot-samples. o-Toluidine is absorbed also through the skin which can contribute significantly to toxicokinetics of the compound. This should be considered when collecting/analysing HBM spot-samples since dermal absorption is slower compared to inhalation exposure. HBM data collected from 24 h urinary samples could better highlight the average exposure but may not be practical. On the other hand, the use of post-shift urinary levels as exposure estimates is likely to rather overestimate than underestimate the exposure since these often represent the peak levels. When compared to the RA based on the measurement of o-toluidine from air, air measurements do not inform us on the effectivity of personal protection of the worker or on the dermal exposure.

In the case of o-toluidine, there is no measured correlation data for external exposure and urinary concentrations. Therefore, BE approach, which is a rather rough method, was selected. This could be further refined by using PBTK models, however there is no specific model available for o-toluidine. Two generic PBK models have been used to investigate the relation between external exposure and urinary values, however the output of both models differed considerably hence it was decided to not use the generated data.

In conclusion, by applying the BE methodology and based on three biomonitoring studies, we found that workers exposed to o-toluidine have a cancer risk of 1:20 000 in the worst-case scenario. The levels were also well below the BOELV set under EU Carcinogens and Mutagens Directive. However, results should be considered carefully because of the limited number of HBM data. There is clearly a need for further biomonitoring data on o-toluidine exposure.

7.1 Recommendations for the regulatory risk assessment

The current exercise gives a good example how biomonitoring data can be used in occupational relevant chemical exposure. Biomonitoring equivalent method can point out a rough estimate of exposure when using post-shift urinary biomonitoring data. In the case of o-toluidine, which is a rapidly metabolised chemical, the best option would be to collect consecutive urinary samples covering a whole day, i.e. 24 h (i.e. pre-shift, during shift, post-shift, evening and next morning). The data on consecutive periods of production of urine would be very informative for parameterising a PBTK model in the future.

7.2 Future prospects

In future, risk assessment would benefit from well-designed biomonitoring studies. In addition, proper toxicokinetic correlation studies for controlled o-toluidine air concentration and urine excretion in humans can benefit future modelling. Moreover, exposure assessment by biomonitoring o-toluidine haemoglobin adducts can highlight exposure for longer and cumulative time periods. Human metabolism and kinetics of o-toluidine should be studied further.

Development of a specific o-toluidine PBTK model would benefit future risk assessments, especially with regards to exposure reconstruction modelling. In addition, developing an adverse outcome pathway (AOP) for human bladder carcinoma would be of benefit also for risk assessments of other arylamine-type chemicals which have similar adverse outcome.

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Annex B: cadmium risk assessment

D5.5 Substance-group specific risk assessment for Cadmium

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1 Introduction

Cadmium (Cd) is a toxic metal widely distributed in the environment. It is naturally abundant but enriched through e.g. industrial and agricultural activities. Measured levels of Cd in agricultural products vary widely, depending on plant varieties, soil types, growing conditions and agricultural methods. Anthropogenic sources, including smelter emissions and the application of fertilizers and sewage sludge to land, may contribute to the contamination of both soils and crops. Previous studies indicated that Cd-bearing fertilizers, especially manures, are an important source for Cd entering into soil. Cd is a highly persistent environmental toxicant that exhibits higher rates of soil-to-plant transfer than other toxic heavy metals (e.g. lead or mercury), making Cd a food-chain contaminant of great concern.

The two major sources of Cd exposure identified for the general population are foodstuffs and cigarette smoke. Concerning the major dietary sources of Cd in food available on the European market, EFSA (the European Food Safety Authority) reported that relatively higher levels of Cd were found in algae formulations, cocoa-based products, crustaceans, edible offal, fungi, oilseeds, seaweeds and water molluscs compared to other food (EFSA, 2009a).

Safety limits of Cd in the environment and foodstuffs were established to safeguard population health. Concerning foodstuffs, Regulation (EC) No. 1881/2006 sets Cd maximum levels ranging from 0.050 mg/kg (some meat products and vegetables) to 1.0 mg/kg (kidney from some animals, bivalve molluscs and cephalopods) (EC, 2006). These levels continue to be reviewed by the European Commission, so an updated scientific basis would be of great importance. A safety limit of 3 mg/kg is applied to soils that are used for producing food crops for human consumption, while a 3 µg/L limit is applied to drinking water (IPCS, 1992).

This document focuses on risk assessment and estimation of burden of disease (BoD) based on HBM data. Work contributing to policy questions as: a) is there a link between high soil contamination with Cd and human exposure via dietary sources (see Annex 1) and b) what is the maximum acceptable level for Cd in foodstuff, is ongoing.

2 Methodology

The present document intends to assess the risk associated to Cd exposure, considering the results already obtained especially by EFSA, JECFA and ATSDR. Biomonitoring data were used to improve the risk assessment yet performed. The applied methodology could be summarized as follows:

1) Firstly, a summary of the Cd hazard assessment was produced including identification and assessment of dose-response relationships for the health effects of environmental (low-level) exposure to Cd. Health hazards of Cd have been extensively reviewed in reports by various agencies/recognised bodies, among which the following were considered for this work: the International Agency for Research on Cancer (IARC, 1993), the European Food Safety Authority (EFSA 2009, 2011 and 2012), the Joint Expert Committee on Food Additives and Contaminants (JECFA, 2010), the Agency for Toxic Substances and Disease Registry (ATSDR, 2012), the European Chemicals Agency (ECHA, 2013), the International Union on Pure and Applied Chemistry (IUPAC) (Nordberg et al. 2018) and the French Agency for Food, Environmental and Occupational Health & Safety (ANSES, in prep.).

2) Secondly, exposure assessments as performed in the EFSA's Cd risk assessment reports were summarised, thereby detailing whether biomonitoring data were considered or not.

3) Thirdly, the risk characterisation and the uncertainty analysis by EFSA was described.

4) The use of biomonitoring data to improve the risk assessment was performed.

5) Additionally, a calculation of the Cd attributable BoD was done for certain endpoints.

3 Summary of hazard characterisation

3.1 Hazard classification

IARC classified Cd and Cd compounds as carcinogenic to humans (Group 1) based on sufficient evidence that long-term occupational exposure to Cd contributes to the development of lung cancer (IARC, 1993).

Cd is also classified as Carc. 1B (H350: may cause cancer), Muta. 2 (H341: suspected of causing genetic defects), Repr. 2 (H361: suspected of damaging fertility or the unborn child) and STOT RE 1[3] (H372: causes damage to organs) in the EU Classification, Labelling and Packaging (CLP) regulation (EC) No 1272/2008[4]. The STOT RE 1 classification is based on Cd adverse effects after prolonged exposure on multiple organs, especially on kidney and bone. Because of these particular effects, Cd was identified as Substance of Very High Concern (SVHC) and is included on the Candidate list for authorisation under the EU Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) regulation (ECHA 2013).

[3] STOT RE stands for Specific Target Organ Toxicity - Repeat Exposure i.e. specific target organ toxicity arising from repeated exposure to a substance or mixture.
[4] Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006.

3.2 Hazards and health-based guidance values

Key data used by JEFCA (2010), EFSA (2009) and ATSDR (2012) for the derivation of Cd dietary intake limit values and U-Cd health-based guidance values, based on Cd renal effects are summarized in Table 1. The kidney dysfunction was considered as the critical effect, i.e. the effect that occurs at the lowest exposure. However, increasing evidence for low-dose bone effects from the last years brought the scientific community to consider also bone effects as a possible critical effect (ATSDR, 2012; Nordberg et al., 2018; ANSES, in prep.)

Table 1 - Key data for the derivation of Cd dietary intake limit values and health-based guidance values for U-Cd, based on the renal tubular impairment effect of Cd.

	EFSA (2009)	JECFA (2010)	ATSDR (2012)
Key study	**Pooled analysis from EFSA** **(35 epidemiologic studies)**		**Meta-analysis of environmental exposure studies**
Evaluation of tubular proteinuria & U-Cd excretion	**β2-microglobulin & U-Cd (µg/g crea)** (Exclusively for a population over 50 years of age)		**Low molecular weight proteinuria & U-Cd (µg/g crea)** (selected studies reported a dose-response relationship in sufficient detail so that the dose-response function could be reproduced independently)
Critical U-Cd (µg/g crea)	**1 µg/g crea** ($BMDL_5$ of 4 µg/g crea and specific adjustment factor of 3,9 to account for human variability in U-Cd within each dose-subgroup in the analysis)	**5.24 µg/g crea** (4.94-5.57) (point of gradient change in the slope)	**0.5 µg/g crea** (95% lower confidence limit associated with 10% increased risk of low molecular weight proteinuria)
Dietary Cd assessment model	Adapted from Amzal *et al.*, 2009	Adapted from Amzal *et al.*, 2009 (Cd half-life)	Kjellstrom & Nordberg, 1978
Toxicological reference value (oral)	**TWI** 2.5 µg/kg bw/week **TDI** 0.36 µg/kg bw/day	**PTMI** 2.25 µg/kg bw/month **PTWI** 5.6 µg/kg bw/week	**MRL** 0.1 µg/kg bw/day

PTMI = Provisional Tolerable Monthly intake; PTWI = Provisional Tolerable Weekly Intake; TWI = Tolerable Weekly Intake; TDI = Tolerable Daily Intake; MRL = Minimal Risk Level; Crea = Creatinine

In 2012, the ATSDR re-evaluated the toxicological profile of Cd and lowered its chronic oral Minimal Risk Level (MRL) of 0.2 µg/kg bw/d set in 1999 to 0.1 µg/kg bw/d. This lower chronic oral MRL was calculated from the 95% lower confidence limit of the urinary Cd (U-Cd) level associated with a 10% increased risk of low molecular weight proteinuria (0.5 µg/g crea) estimated from a meta-analysis of selected environmental exposure studies (ATSDR, 2012). Starting from this value (0.5 µg/g crea) as a burden at the age of 55 years[5] and assuming a chronic intake of Cd, an intake of 0.33 µg/kg bw/day for women and 0.70 µg/kg bw/day for men was calculated using a biokinetic multicompartimental PBPK model. Applying a safety factor of 3 to take into account interindividual variation, an MRL of (0.33/3=) 0.1 µg/kg bw/day was established.

ATSDR refers that the environmental Cd exposure studies strongly support the identification of bone and kidney as the most sensitive targets of chronic Cd toxicity. However, the renal effects database being stronger compared to the skeletal effects, it was used for derivation of the chronic oral MRL for Cd.

In 2009, EFSA conducted a systematic review of the scientific literature on the relationship between U-Cd and renal or bone biomarkers of Cd toxicity (EFSA, 2009b). The most frequently studied biomarker was β-2-microglobulin (β2M), for which a benchmark dose (BMD) evaluation was performed. The data were made of 165 matched pairs of group means of U-Cd and level of β2M from 35 different epidemiological studies. EFSA calculated a benchmark dose lower confidence limit for a 5% increase (BMDL5) of the prevalence of elevated β2M equal to 4 µg Cd/g crea. A chemical specific adjustment factor of 3.9 accounting for human variability in U-Cd was applied to the BMDL5, generating a critical U-Cd concentration of 1 µg/g crea (at age above 50 years). The dietary Cd intake corresponding to this critical U-Cd concentration after 50 years of exposure was then estimated using a one-compartment model. This model developed by Amzal et al. (2009) fitted to a large data set based on non-smoking Swedish women (at age range 58 to 70 years)[6] comprising measurement of dietary Cd exposure and U-Cd concentration to allow an estimation of the relationship between both. In order to remain below the critical U-Cd of 1 µg Cd/g crea in 95 % of the population by age 50, the maximum average daily dietary Cd intake was found to be 0.36 µg Cd/kg bw/day, corresponding to a weekly dietary intake of 2.52 µg Cd/kg bw/week. EFSA thus established a tolerable weekly intake (TWI) for Cd of 2.5 µg/kg bw/week (EFSA, 2009a).

Based on EFSA's meta-analysis of epidemiological studies, JEFCA derived a health-based guidance value of 5.24 µg Cd/g crea (JEFCA, 2010), thus 5-fold higher than the health-based guidance value of 1 µg Cd/g crea set by EFSA in 2009. A comparison of the approaches taken by both committees was performed in term of assumptions, starting point and methodology (EFSA, 2011). In light of data associating U-Cd levels below 5.24 µg/g crea with numerous adverse health effects (e.g. chronic kidney disease and type-2 diabetes), it was concluded that JEFCA-established safe intake guideline and U-Cd threshold limit would need to be reassessed (Satarug et al., 2017).

[5] 55 years is the approximate age of peak Cd concentration in the renal cortex associated with a constant chronic intake (ATSDR, 2012)

[6] The model calculations takes into consideration the human variability in Cd absorption rates (1–10 %), so that high absorption rates common in women of reproductive age groups due to high prevalence of low and empty iron stores as well as variations in half-life were included.

Thus, the ATSDR, JEFCA and EFSA's latest Cd health risk assessment reports are based on human biomonitoring (HBM) data regarding establishment of the dose-response relationship (measurements of U-Cd concentration and urinary concentration of β2M as biomarker for renal impairment).

The German HBM Commission retained the critical U-Cd concentration of 1 µg/g crea set by EFSA for setting the HBM-I value[7] for adults (Bundesgesundheitsbl. 2011). For reasons of practicability however, the HBM-I value was set at 1 µg/L (instead of 1 µg/g crea), supported by the similarity of Cd levels based on creatinine excretion and urinary volume from the German Environmental Surveys (Schulz et al., 2011). The HBM-I value in children and adolescents was set at half the value for adults (HBM-I of 0.5 µg/L) in order to take into account the accumulation of Cd with age (Schulz et al., 2011).

Under HBM4EU, this critical U-Cd concentration of 1 µg/g crea based on the increase in prevalence of elevated β2M urinary levels was selected as HBM guidance value (HBM-GV) for the general population. However, as a renal tubular dysfunction can come from substantial accumulation of Cd in the kidney cortex after long-term Cd exposure, a life-time PBPK model[8] (ANSES, in prep.) was used to determine U-Cd concentrations at each age leading to reach 1 µg Cd/g crea at age 55-60 years, considering a constant Cd dietary intake (here 0.72 µg/kg bw/day by using the ANSES biokinetic PBPK model[9]) (Figure 1). These predicted limit concentrations of U-Cd according to ages (Annex 1) can be seen as alert levels, considering the increase of Cd body burden with age and its accumulation in the kidneys.

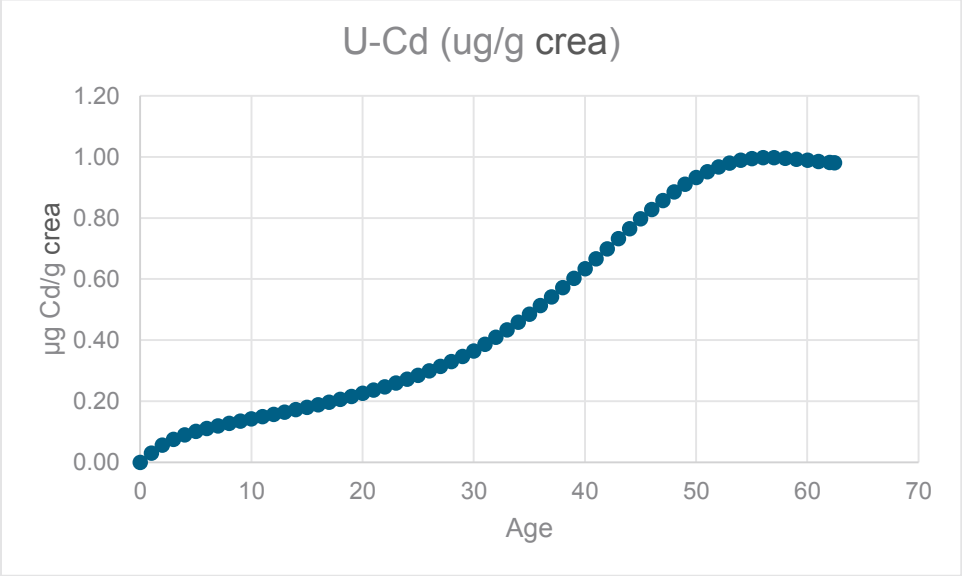
[7] The HBM-I values of the HBM Commission represents the concentration of a substance in human biological material below which - according to the knowledge and judgement of the HBM Commission - there is no risk for adverse health effects and, consequently, no need for action.

[8] Taking into account changes in bodyweight and creatinine excretion according to age

[9] The TK/PBPK model input parameters of Cd leading to a selected U-Cd threshold at a certain age (i.e. 1 µg Cd/g crea at age 55-60 years in this case) is strongly dependent of the model structure and biological characterisation, associated to chemical-specific parameters. EFSA used the TK one compartment model at constant bodyweight from Amzal et al., 2009 to estimate the dietary cadmium exposure that corresponds to the U-Cd concentration of 1 µg/g crea after 50 years of exposure. The modelling results indicated that in order to remain below 1 µg Cd/g creat in urine in 50% / or 95% of the population by age of 50, the long term daily dietary Cd exposure should not exceed respectively 0.78 / 0.36 µg Cd/kg bw and the weekly dietary Cd exposure should not exceed respectively 5.46 / 2.52 Cd/kg bw (this latest value being the recommended TWI EFSA, 2009). The mean Cd half-life considered in the model is about 11.6 years.

The ANSES PBPK model (ANSES, in prep.) is based on the 8 compartments model of Kjellstrom and Norberg (1978) and was further implemented by introducing equations describing the mean body weight and creatinine excretion evolutions according to age (based on French general population data). The mean Cd half-life considered in this model is about 16 years. The estimated Cd clearance is thus lower than the estimated Cd considered in the TK model used by EFSA. These differences between the two models leads to different input parameters (Cd intake value) needed to reach the U-Cd threshold value of 1 µg Cd/g crea at age 5-60 years).

Figure 1: Cadmium urinary concentration (µg/g crea) as a function of age (year), assuming a constant dietary intake (0.72 µg Cd/kg bw/day according to the ANSES biokinetic PBPK model used).



4 Summary of available exposure assessment

A term of reference specified in the EU Commission's request to EFSA for a scientific opinion on the risks to human health related to the presence of Cd in foodstuffs was that available HBM data had to be taken into account when assessing the exposure, by comparing these results with the calculated exposure. Therefore, in 2009, EFSA summarised in its opinion published HBM data referring to the general population, children in hot-spot areas or high-mollusc consumers from France, Poland, Czech Republic, the United States, Germany, Belgium, Sweden, United Kingdom and Canada (EFSA, 2009a). To assess the Cd dietary exposure however, occurrence data and consumption data reported in the EFSA's Concise European Food Consumption Database were used (national food consumption dietary surveys were considered to estimate the consumption pattern of specific sub-groups such as vegetarians and children). The mean dietary exposure across EU countries was estimated to be 2.3 µg/kg bw/week with a maximum estimated at 3.0 µg/kg bw/week.

In 2012, EFSA revised its Cd exposure assessment based on updated Cd occurrence data in food and extended consumption data (EFSA, 2012). Average middle bound lifetime Cd dietary exposure for the EU population as a whole (including infants, toddlers, children, adolescents, adults, elderly and very elderly) was estimated at 2.04 µg/kg bw/week[10]. It was highest in toddlers with an average of 4.85 µg/kg bw/week and lowest in the elderly population group at 1.56 µg/kg bw/week.

[10] Individual dietary survey results varied between a minimum lower bound mean of 1.15 and a maximum upper bound of 7.84 µg/kg bw/week and a minimum 95th percentile lower bound of 2.01 and a maximum upper bound of 12.1 µg/kg bw/week, reflecting different dietary habits but also likely differences in survey methodologies and the countries covered for the different age classes.

5 Risk characterisation and uncertainty analysis

In 2009, EFSA performed a risk characterisation for dietary Cd exposure by comparing the calculated dietary Cd exposures of subgroups of the EU general population with the recommended TWI of 2.5 µg/kg bw (EFSA, 2009a). Conclusions were that the mean Cd exposure for adults across Europe was close to, or slightly exceeding the TWI of 2.5 µg/kg bw/week. The exposure of some subgroups of population, such as vegetarians, children, smokers and people living in highly contaminated areas was determined to exceed the TWI by about 2-fold.

Based on revised Cd dietary exposure assessment from 2012, EFSA indicated that the conclusions drawn from the 2009 risk assessment were confirmed. Also, that the actual risk of adverse effects for an individual at current dietary exposure in the EU was low for adults, because the TWI was established based on an early indicator of changes in kidney function suggesting possible kidney damage later on in life. Nevertheless, and considering the proximity of TWI and exposure determined, EFSA concluded that there was a need to reduce exposure to Cd at the population level.

6 Improving the RA by biomonitoring

In order to confirm the need to reduce exposure to Cd at the population level, a risk assessment was performed, either:

Case-study 1) based on estimations of the fraction of women from the EU general population exceeding the HBM-GV of 1 µg Cd/g crea at age above 50, or

Case-study 2) based on estimations of the fraction of women from the EU general population at risk of exceeding the HBM-GV when reaching the age of 55-60 years, when considering their U-Cd concentrations at earlier ages.

Based on HBM data, calculations of the Cd attributable disease burden (ADB) regarding chronic kidney disease (CKD) and osteoporosis at total hip or spine in women aged above 50 years in the EU was also attempted in this report.

6.1 Evaluation of the available biomonitoring data

6.1.1 Selection of relevant HBM datasets

Several published HBM datasets having explored U-Cd levels in the EU general population are available. Conditions for selecting specific HBM datasets were the following:

1) Percentiles of the U-Cd levels among the individuals had to be indicated, to allow for reconstructing the U-Cd distribution and thereby assessing the percentage of the selected subgroup of population exceeding the HBM guidance value;

2) The individuals who provided the samples for the HBM measurements (i.e. U-Cd concentrations) had to correspond in terms of gender, age, type of exposure to Cd to the specific population on which exposure-response relationships were identified for the ADB calculations (explicitly for case-study 1) (see section 6.3).

6.1.2 Description of the selected HBM datasets

The HBM results of the BIOAMBIENT_ES study conducted in Spain (in 2009) and the ENNS study conducted in France (in 2006-2007) were selected (López-Herranz et al., 2016; Fréry et al., 2011) for case study 1. The percentiles for U-Cd women aged 50-65 years for the

BIOAMBIENT_ES study (N=119) and 60-74 years in the ENNS study (N=421) were available, allowing for direct estimation of the ADB based on the HBM exposure data measured in these women, without making any assumption regarding their future Cd intake and kidney accumulation. The ADB calculated in this first case study represents the actual number of hip and spine osteoporosis and CKD cases in women aged above 50 in Spain and France attributable to their past Cd exposure.

The HBM results from the DEMOCOPHES study were also selected, as the percentiles of U-Cd levels in women from 16 EU countries were available, which gives an unique opportunity to have an insight of the ADB at EU level, based on Cd country-specific urinary levels (case study 2). However, the women in the DEMOCOPHES study were aged 18 to 45 years (Den Hond et al., 2015). Yet, publications of the U-Cd levels results were available for the following age ranges: 18-35 years, 35-40 years and >40-45 years (Berglund et al., 2015). Thus, in order to calculate the ADB related to hip or spine osteoporosis, assumptions regarding the Cd intake of these women from their age until they would reach 50 years were needed. The ADB calculation in this case study is therefore representing the possible Cd exposure attributable number of hip or spine osteoporosis cases in EU women in the next years, depending on their Cd intake after their U-Cd level was measured in the DEMOCOPHES study.

6.2 Risk assessment for the sample populations

6.2.1 Risk assessment based on the BIOAMBIENT_ES and ENNS results

The distribution of Cd concentrations in the urinary samples collected in the BIOAMBIENT_ES and ENNS studies were reconstructed based on the percentiles. The fractions of urinary samples (collected in women aged > 50 years) exceeding the U-Cd threshold value of 1 µg/g crea (EFSA critical Cd urinary level (EFSA, 2009); German HBM-I (Bundesgesundheitsbl. 2011); HBM4EU HBM-GV (coming publication)) are indicated in Table 2 for both studies.

Table 2 - Distribution of the U-Cd concentrations measured in women aged above 50 years from the BIOAMBIENT_ES and ENNS studies and percentage of exceedance of the U-Cd threshold value for renal effects (1 µg Cd/g crea)

Study (Country)	Age of participants (women), years	N	U-Cd concentrations (µg/g crea)								% > 1 µg/g crea
			GM	95% CI GM	P10	P25	P50	P75	P90	P95	
BIOAMBIENT_ES (Spain)	50-65	119	0.42	0.34-0.52	-	0.29	0.46	0.69	1.27	1.82	17.4%
ENNS (France)	60-74	421	0.43	0.4-0.46	0.20	0.29	0.42	0.65	0.99	1.15	8.0%

From the **Spanish BIOAMBIENT.ES study**, 17.4 % women aged 50-65 years are exceeding the U-Cd threshold of 1 µg/g crea; from the **French ENNS study**, 8.0% women aged 60-74 years are exceeding the U-Cd threshold of 1 µg/g crea.

A Risk Characterisation Ratio (RCR), corresponding to the ratio between the 95th percentile of the Cd urinary concentrations (HBM data) retrieved in the selected HBM datasets and the selected HBM-GV can be calculated for both HBM datasets:

$$RCR_{internal} = \frac{95\text{th Percentile of HBM results distribution}}{\text{Selected internal threshold (HBM-GV of 1 µg/crea)}}$$

$RCR_{internal\ (BIOAMBIENT\_ES)} = \frac{1.82}{1} = 1.82$ $RCR_{internal\ (ENNS)} = \frac{1.15}{1} = 1.15$

A RCR below 1 means that there is no risk of adverse health effects based on current knowledge. A value above 1 is an indicator, a raised flag, that the risk is increased, not meaning that health effects are already present, seeing e.g. the uncertainties and safety factors applied. It is an indicator that there is a concern of the exposure with regard to health-based criteria and is signal for policy-makers.

6.2.2 Risk assessment based on the DEMOCOPHES results

A life-time PBPK model was used to identify the "alert" levels of U-Cd at each age which lead to reach the U-Cd HBM-GV of 1 µg/g crea at age 55-60 years (Annex 2), considering a constant dietary intake of Cd (here 0.72 µg/kg bw/day, according to the ANSES biokinetic PBPK model used). The mean U-Cd "alert" levels for the age ranges 35-40 years and 41-45 based on these simulations are presented in Table 3.

Table 3: Mean U-Cd alert values according to age ranges, based on a constant dietary intake (here at 0.72 µg Cd/kg bw/day)

Age range (years)	Estimated bodyweight (kg)	Mean alert U-Cd levels*
35-40	73	**0.51 µg/g crea**
41-45	73	**0.70 µg/g crea**

** if the U-Cd concentration is higher than the alert value at this age category, the threshold of 1 µg Cd/g crea will be exceeded at the age of 55-60 years.*

The distributions of Cd concentrations in the urinary samples collected in the DEMOCOPHES study were reconstructed for 14 participating countries, based on the percentiles (Cyprus and UK were excluded because of the low numbers of samples; no Cd data available for Germany). The fractions of urinary samples collected in women aged 35-40 years and 41-45 years exceeding the alert U-Cd value for their age range (respectively 0.51 Cd µg/g crea and 0.70 µg Cd/g crea), are indicated in Table 4.

Table 4: Distribution of the U-Cd concentrations measured in women from the DEMOCOPHES studies (for 14 participating countries) and percentage of exceedance of the mean U-Cd alert level for renal effects calculated for each age range (35-40 years = 0.51 µg Cd/g crea; 41-45 years = 0.70 µg Cd/g crea).

Country	Age (years)	N	U-Cd concentrations (µg Cd/g crea)								% > U-Cd alert level
			GM	95% CI GM	P10	P25	P50	P75	P90	P95	
Belgium	**35-40**	58	0.182	0.153 - 0.217	0.102	0.116	0.173	0.311	0.414	0.541	5.90%
	41-45	45	0.187	0.151-0.233	0.091	0.144	0.208	0.267	0.371	0.462	0.80%
Czech Rep	**35-40**	56	0.220	0.200 - 0.250	0.130	0.170	0.220	0.290	0.430	0.470	3.70%
	41-45	24	0.230	0.190-0.270	0.170	0.190	0.220	0.280	0.420	0.420	0.10%
Denmark	**35-40**	55	0.105	0.860 - 0.129	0.045	0.070	0.111	0.170	0.254	0.385	2.10%
	41-45	74	0.134	0.111-0.162	0.044	0.088	0.147	0.252	0.320	0.417	0.90%
Spain	**35-40**	49	0.215	0.173 - 0.267	0.093	0.136	0.192	0.389	0.580	0.638	10.9%
	41-45	57	0.223	0.186-0.269	0.090	0.138	0.215	0.321	0.575	0.748	6.40%
Hungary	**35-40**	45	0.160	0.140 - 0.200	0.060	0.120	0.160	0.270	0.310	0.440	1.50%
	41-45	23	0.130	0.100-0.180	0.050	0.110	0.140	0.190	0.260	0.440	0.30%
Ireland	**35-40**	41	0.247	0.199 - 0.306	0.089	0.153	0.279	0.405	0.559	0.658	12.1%
	41-45	45	0.325	0.268-0.394	0.116	0.172	0.341	0.490	0.715	0.933	11.5%
Lux	**35-40**	34	0.207	0.170 - 0.252	0.119	0.135	0.23	0.345	0.433	0.589	11.2%
	41-45	19	0.225	0.187-0.270	0.102	0.172	0.213	0.281	0.340	0.361	0.00%
Poland	**35-40**	44	0.371	0.311 - 0.442	0.125	0.202	0.409	0.620	0.927	1.057	32.5%
	41-45	24	0.492	0.353-0.685	0.182	0.345	0.468	0.947	1.343	1.479	30.3%
Portugal	**35-40**	52	0.156	0.131 - 0.186	0.071	0.111	0.161	0.225	0.361	0.440	3.40%
	41-45	29	0.225	0.185-0.273	0.094	0.158	0.261	0.308	0.439	0.545	0.20%
Romania	**35-40**	46	0.170	0.136 - 0.212	0.053	0.136	0.178	0.279	0.406	0.681	10.9%
	41-45*	7	0.148	0.060-0.370	0.017	0.063	0.241	0.375	0.955	0.955	16.1%
Sweden	**35-40**	44	0.124	0.106 - 0.144	0.069	0.088	0.132	0.177	0.234	0.246	0.10%
	41-45	33	0.161	0.133-0.195	0.086	0.118	0.168	0.208	0.288	0.411	0.00%
Slovenia	**35-40**	48	0.222	0.191 - 0.258	0.134	0.16	0.229	0.337	0.422	0.509	4.80%
	41-45	46	0.252	0.206-0.307	0.115	0.189	0.286	0.383	0.530	0.545	0.60%
Slovak Rep	**35-40**	51	0.220	0.195 - 0.250	0.116	0.158	0.225	0.311	0.382	0.454	3.10%
	41-45	29	0.290	0.250-0.342	0.144	0.234	0.295	0.436	0.511	0.514	0.00%
Switzerland	**35-40**	38	0.207	0.183 - 0.233	0.119	0.166	0.202	0.288	0.383	0.411	2.30%
	41-45	38	0.207	0.183-0.233	0.119	0.166	0.202	0.288	0.383	0.411	0.00%

*The number of individuals is limited.

From the data in the tables above it's clear that the countries Spain, Ireland, Poland and Romania in both the age categories (35-40y and 41-45y) exceeded the age specific limit value (corresponding to the HBM-GV of 1 µg Cd/g crea at age 55-60) with more than 5%[11]. This means that the P95 of exposure divided by the HBM-GV (or the RCR) is >1. This observation needs to be considered with caution seeing the strong hypothesis following the application of the PBPK model for lifetime exposure to obtain age-specific U-Cd alerts levels. For example, a constant amount of dietary intake of Cd was assumed over life and was set identical for all EU countries.

[11] The Cd dietary exposure risk assessment conducted by EFSA (EFSA 2009, 2012) concluded that children and consumers at the 95th percentile exposure could exceed the health-based guidance value (TWI of 2.5 µg/kg bw/week). The characterisation of the risk in this present work was also based on the 95th percentile exposure, to be consistent with the EFSA risk assessment.

6.3 Estimation of the attributable disease burden based on the exposure estimates and size of the population exposed to specified levels

6.3.1 General approach

To estimate the burden caused by Cd as a risk factor, information on the population exposure, the exposure-response relationship for a specific effect considered, as well as the background incidence for this effect (in case the exposure-response function is given by a relative risk (RR)) are needed. In the present report, the exposure assessment of population is based on HBM data. The global methodology used is displayed in Figure 2.

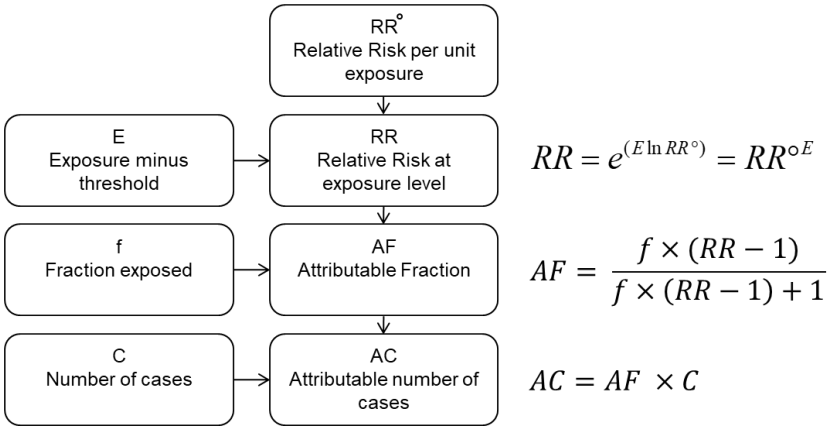


Figure 2: Schedule of methodology applied

From an epidemiological study, the function of the exposure-response relationship expressed per unit of exposure (e.g. an RR or an Odds Ratio (OR)) is converted to an RR at the exposure level that is exceeding an internal health-based guidance value (corresponding to the maximal internal exposure expected to be without appreciable health risk). The exposure-response relationship was in this schedule assumed to be log-linear.

This calculated RR at exposure level above the threshold value is used to derive the attributable fraction (AF), defined as "a measure that quantifies the proportion of BoD that can be attributed to the exposure", which is obtained by applying the function of the exposure-response relationship to the exposures percentiles from the HBM studies. This AF is then applied to the background incidence of the disease (or total number of cases) occurring in the same population group the exposure-relationship was identified in, to estimate the attributable incidence (or attributable cases).

6.3.2 Identification of dose-relationships for selected critical effects

6.3.2.1 Dose-relationship for kidney effects

Regarding the threshold value considered without appreciable risk for the renal effects, the value of 1.0 µg Cd/g crea at age above 50 years was chosen, in agreement with EFSA's 2009 health risk assessment report.

Cd-induced renal toxicity is characterized by tubular proteinuria, i.e. an elevated excretion of low molecular weight proteins (e.g. β2M and Retinol Binding Protein (RBP)) due to damage to the renal tubules. But glomerular damage may also occur, manifested by reduced

glomerular filtration rate (GFR). *In vitro* studies have suggested that Cd could directly affect the glomerulus at low concentration, causing contraction of cultured glomeruli and associated vascular cells (Barrouilet et al., 1999; Hirano et al., 2005). Another possibility is that autophagy and apoptosis are induced in intraglomerular mesangial cells, glomeruli are injured and, consequently, GFR is reduced (Orr and Bridges, 2017). Associations have been also reported between low-level Cd exposure and indicators of glomerular function in cross-sectional analyses (Akesson et al., 2005; Suwazono et al., 2006; Hwangbo et al., 2011; Kim et al., 2015).

A negative association between U-Cd and GFR was observed by Akesson et al. (2005), based on measurements of U-Cd and markers of renal injury in 816 Swedish women aged 53-64 years. Exposure of the women to Cd via the general diet was at a low Cd exposure level (mean U-Cd concentration of 0.67 µg/g crea). A regression slope between U-Cd and the GFR was calculated from this study, consisting in a decline by 7.9 ml/min of the GFR per µg/L of U-Cd.

Ginsberg et al. (2012) converted this reported regression slope to units that could be related back to the definition of CKD (ml/min/1.73m^2) and adjusted it to µg/g crea. This yields a slope of 5.8% decrease in GFR per µg Cd/g crea in urine.

Since clinical symptoms usually do not present in individuals with only tubular dysfunction (proteinuria) (Bernard, 2004), it is not suitable to use tubular dysfunction as an endpoint for estimating the BoD of Cd exposure related to the renal effects. The endpoint chosen for further analysis was decreased GFR, because it has a direct clinical relevance to CKD.

CKD is defined as an estimated glomerular filtration rate (eGFR) below 60 mL/min/1.73 m^2 or urinary albumin to creatinine ratio above 30 mg/g (Satarug et al., 2018). It is categorized in five stages that are mainly based on the GFR, according to the National Kidney Foundation guideline that has been accepted worldwide (National Kidney Foundation, 2002). The early stages (stages 1–2) of CKD usually do not show clinical symptoms, and the disease may only be detected in urine/blood tests. In stage 3 most of the patients will not show symptoms but some may have swelling of hands and feet, back pain, abnormal amounts of urine. Health effects related to the waste build up in the body are high blood pressure, anaemia and bone disease. The late stages of CKD (stage 4–5), characterized by severe decreases in the GFR (stage 4: 15–30 ml/min/1.73m^2; stage 5: <15 ml/min/1.73m^2) requires clinical interventions such as dialysis or a kidney transplant (Zang et al., 2019). Late stage CKD constitutes a large and worldwide public health burden.

6.3.2.2 Dose-relationship for bones effect

Statistically significant ORs for risk of osteoporosis were identified from a population-based study among 56- to 69-year old women with low environmental Cd exposure (Engström et al., 2011). In this study, U-Cd was assessed in 2688 women within the Swedish Mammography Cohort (1997). The limit of detection (LOD) in the study was equal to 0.002 µg Cd/L and the 5th percentile of all samples equal to 0.15 µg Cd/g crea.

Categories of U-Cd 0.50-0.75 µg/g crea and ≥0.75µg/g crea were compared to the U-Cd category <0.50 µg/g crea. A 40% to 60% increased risk of osteoporosis, per 0.42 µg/g crea increment of U-Cd, at the femoral neck, total hip and lumbar spine was determined, with no observed threshold. Statistically significant multivariable adjusted ORs for the risk of hip or spine osteoporosis were observed: 1.43 (95% CI 1.15-1.78) per 0.42 µg Cd/g crea in all women and 1.55 (95% CI 1.05-2.29) per 0.42 µg Cd/g crea among never-smokers women (Table 7). For urinary excretion of Cd in the range 0.5 and 0.75 µg/g crea, the OR for risk of

hip or spine osteoporosis equals 1.61 (95% CI 1.20-2.16) in all women and above or equal to 0.75 µg/g crea of urinary excretion of Cd, the OR for risk of hip or spine osteoporosis in all women is 1.95 (95% CI 1.30-2.93).

The higher risk of hip or spine osteoporosis in all women above 56 years of age was selected to perform the Cd exposure ADB calculations related to bones effects.

Therefore, the value of 0.5 µg Cd/g crea was considered as the U-Cd threshold value (<0.5 U-Cd µg/g is the reference category from the Engström et al., 2011 study), i.e. meaning that no adverse effects related to osteoporosis would take place below it. Also in NHANES, women ≥ 50 years of age with U-Cd levels between 0.50 and 1.00 µg/g crea were at greater risk for hip-BMD–defined osteoporosis, relative to those with levels ≤ 0.50 µg/g (Gallagher et al., 2008).

Table 5: Risk of osteoporosis and its 95% confidence interval (CI) at the femoral neck, total hip, lumbar spine and hip or spine in all women and in the group of never-smokers (Engström et al., 2011).

	ORs (95% CI)	
	All women	**Never-smokers**
Femoral neck	n=216	n=101
U-Cd per 0.42 µg/g crea [a]	**1.58 (1.22-2.05)**	**1.95 (1.21-3.16)**
U-Cd, categories		
<0.50 µg/g crea	1.00 (ref)	1.00 (ref)
0.50-0.75 µg/g crea	2.17 (1.51-3.11)	2.09 (1.12-3.93)
≥0.75 µg/g crea	2.45 (1.51-3.97)	3.47 (1.46-8.23)
p trend [b]	< 0.001	0.001
Lumbar spine	n=267	n=141
U-Cd per 0.42 µg/g crea [a]	**1.41 (1.10-1.81)**	**1.35 (0.86-2.12)**
U-Cd, categories		
<0.50 µg/g crea	1.00 (ref)	1.00 (ref)
0.50-0.75 µg/g crea	1.30 (0.91-1.86)	1.17 (0.64-2.15)
≥0.75 µg/g crea	1.97 (1.24-3.14)	3.26 (1.44-7.38)
p trend [b]	0.003	0.13
Hip or spine	n=400	n=201
U-Cd per 0.42 µg/g crea [a]	**1.43 (1.15-1.78)**	**1.55 (1.05-2.29)**
U-Cd, categories		
<0.50 µg/g crea	1.00 (ref)	1.00 (ref)
0.50-0.75 µg/g crea	1.61 (1.20-2.16)	1.27 (0.75-2.14)
≥0.75 µg/g crea	1.95 (1.30-2.93)	4.24 (1.99-9.04)
p trend [b]	< 0.001	< 0.001

[a] To convert OR per 2 SD (0.42 µg/g of crea) increment in U-Cd to per 1 µg/g of crea; $e^{(\ln OR)/0.42}$

[b] linear trend across categories were tested using the median U-Cd within categories as a continuous variable

The RR (relative risk) can be estimated from the OR (odds ratio) based on the following equation (Zhang and Yu, 1998; Hauser et al., 2015):

Equation (1) RR = OR / ((1-baseline prevalence of disease) + (baseline prevalence*OR))

The baseline prevalence is the prevalence without exposure. In our calculation we applied the observed prevalence in the population (exposed and non-exposed), but this will only marginally affect the results. In reality the baseline prevalence of disease of the non-exposed could be somewhat lower, thus the RR higher, meaning that the calculated ADB could be underestimated.

6.3.3 Attributable disease burden calculation

6.3.3.1 Kidney effects

The review of the GFR baseline data in the general, healthy population was conducted by Zang et al. (2019) (Table 6). Data about GFR were available for France and Spain for which also HBM data were present (BIOAMBIENT_ES and ENNS; see Table 2). The following information was recorded: the mean and the standard deviation (SD) of the published GFR, and the mean or median age of the population from which the GFR data was collected. Information about age was collected because GFR decreases by age, as a physiological process, which should be considered.

Table 6: Mean GFR in Spain and France (Zang et al., 2019)

Country	GFR, mean (SD) (ml/min/1.73 m^2)	Mean Age (years)	Reference
Spain	84.6 (36.7)	49.5	Otero et al., 2010
France	71.0 (15.0)	68.3	Bacchetta et al., 2010

The GFR generally follows a normal distribution in the general human population (Glassock and Winearls, 2009), thus the prevalence of CKD stage 3 (30-59 ml/min/1.73m^2), stage 4 (15–30 ml/min/1.73m^2) and stage 5 (<15 ml/min/1.73m^2) can be modelled using the cumulative density function from a normal distribution given the mean and the SD.

6.3.3.1.1 Estimation of the age-specific CKD prevalence

In general, after age 30-40, the GFR declines by about 0.8 ml/min/1.73m^2 per year in healthy populations (Glassock and Winearls, 2009). Assuming that this rate of decline is consistent through life after 40 years old and using a published mean GFR (x_α) obtained for a population with an average age of α, the mean GFR for an older population (57.5 years, mean value of range 50-65 years, Table 2) of the same geographic area ($x_{\alpha+n}$) can be modelled using Equation 2 below:

Equation (2) $$X_{\alpha+n} = X_\alpha - 0.8\ n$$

where n is the number of years that the calculated population is older than the published population.

The estimation of the age-related GFR decrease for the Spanish population is presented in Table 7.

Table 7: Age-related GFR decrease for the Spanish population: GFR distribution at mean age 57.5 years (calculations with Equation 2).

Age in years (mean age of BIOAMBIENT_ES, women)	Age-related GFR decrease (mean ml/min/1.73 m2)
57.5	78.2 (SD 36.7)

The prevalence of CKD stages 3, 4 and 5 based on the normal distribution of the GFR in the Spanish population at mean age of 49.5 years (mean GFR of 84.6 ml/min/1.73 m^2 and SD of 36.7) and in the BIOAMBIENT_ES population at mean age of 57.5 years (Figure 3; mean GFR of 78.2 ml/min/1.73 m^2 and SD of 36.7) years are presented in Table 8.

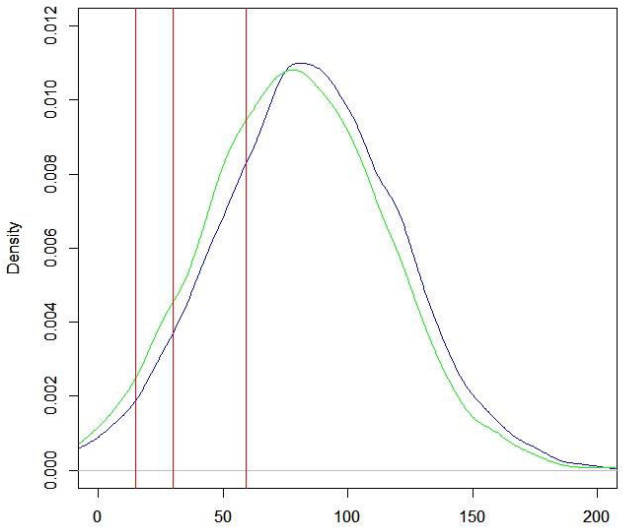


Figure 3: Age-related GFR decrease for the Spanish population (green curve). The red vertical lines present the cut-off values for CKD stages 3, 4 and 5.

Table 8: Prevalence of CKD according to the GFR in Spain and BIOAMBIENT_ES, at mean age 49.5 and 57.5 years, respectively.

GFR (ml/min/1.73m^2)	Mean age (years)	0-15 (CKD 5)	15-30 (CKD 4)	30-59 (CKD 3)	0-59 (CKD 3, 4 and 5)
Spain (BIOAMBIENT.ES)	49.5	2.89%	3.94%	17.43%	24.27%
	57.5	4.25%	5.20%	20.59%	30.04%

6.3.3.1.2 Cd-related CKD prevalence based on Cd exposure in conjunction with aging

Based on the epidemiological data, U-Cd at 1 µg/g crea was used as the threshold value below which no clinically significant adverse renal effects are likely to occur. Hence, if the mean baseline GFR for a reference population (x) is known, the mean GFR for a population with given Cd exposure (measured by U-Cd) can be described as:

Equation (3)	$X_{cd} = x\ (1\text{-}0.058(U\text{-}Cd\text{-}1))$

Based on Equations (2) and (3), the mean GFR for a population under the influence of aging and exposure to Cd can be described as:

Equation (4)	$X_{\alpha+n,cd} = (X_{\alpha}\text{- } 0.8\ n)(1 - 0.058\ (U\text{-}Cd\text{-}1))$

The following tables and figures present the calculations for the BIOAMBIENT study. Decreased GFR due to age and Cd exposure was estimated at two exposure levels: the P90 (1.27 µg Cd/g crea) and the P95 (1.82 µg Cd/g crea) (Table 2). Similar calculation were performed of the French study (ENNS).

Table 9:Age and Cd exposure-related decrease in GFR, with U-Cd concentrations at the P90 and P-95 U-Cd concentrations of the BIOAMBIENT_ES study (calculations with Equation 4).

Spain	Age and Cd exposure-related GFR decrease (ml/min/1.73 m^2)
	Age 57.5 (mean age of BIOAMBIENT_ES women)
P90 U-Cd concentration (1.27 µg/g crea) of the BIOAMBIENT_ES study	76.97 (SD 36.7)
P95 U-Cd concentration (1.82 µg/g crea) of the BIOAMBIENT_ES study	74.5 (SD 36.7)

Table 10: Prevalence of CKD according to age and P90 Cd exposure-related GFR decrease in Spain.

GFR (ml/min/1.73m^2)	0-15 (CKD 5)	15-30 (CKD 4)	30-59 (CKD 3)	0-59 (CKD 3, 4 and 5)
Spain (BIOAMBIENT.ES) P90 U-Cd	4.57%	5.46%	21.77%	**31.22%**
Spain (BIOAMBIENT.ES) P95 U-Cd	5,25%	6,02%	24.19%%	**33.64%**

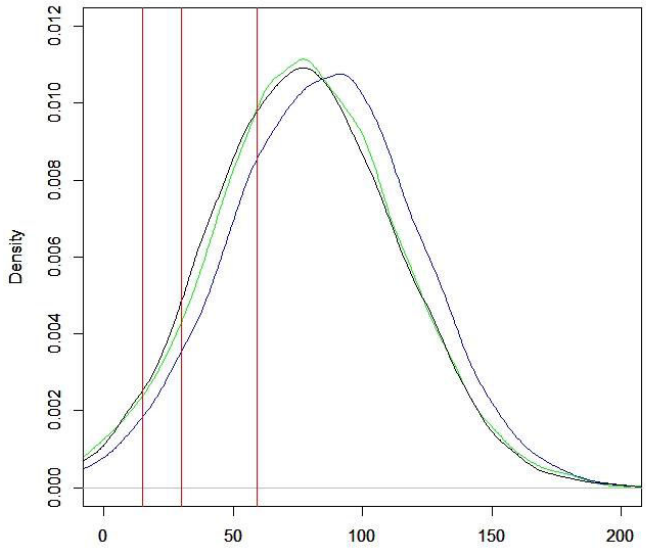


Figure 4: Age and Cd exposure-related GFR decrease - Spain with P90 U-Cd concentration of the BIOAMBIENT_ES study (black curve).

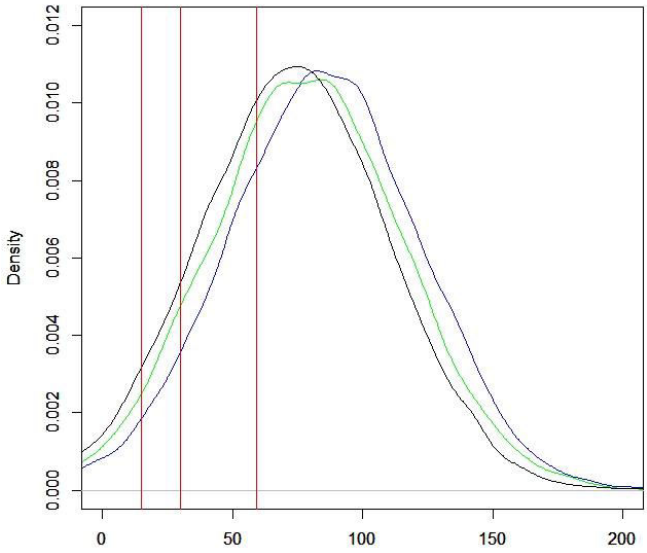


Figure 5: Age and Cd exposure-related GFR decrease - Spain with P95 U-Cd concentration of the BIOAMBIENT_ES study (black curve).

6.3.3.1.3 Cd exposure AF of CKD stage 3 to 5 in Spain

Table 11 below presents the CKD prevalence due to Cd exposure (at P90 and P95).

Table 11: CKD prevalence due to Cd exposure (P90 and P95 of U-Cd).

Shift in CKD prevalence due to P90 Cd exposure level. This % does not take into account that only 5% of the persons (P90 to P95) is exposed to this level.				
GFR (ml/min/1.73m^2)	**0-15 (CKD 5)**	**15-30 (CKD 4)**	**30-59 (CKD 3)**	**0-59 (CKD 3, 4 and 5)**
Spain	**0.31%**	**0.26%**	**1.18%**	**1.18%**
Shift in CKD prevalence due to P95 Cd exposure. This % does not take into account that only 5% of the persons (P90 to P95) is exposed to this level.				
Spain	**1.00%**	**0.82%**	**3.60%**	**3.60%**
Total AF of CKD cases due to P90 (5% of the population) and P95 Cd exposure (5% of the population) for Spain. This considers the percentage of the population exposed to Cd at the P90 and P95.				
Spain	**0.07%** (= 0.31% × 5% + 1% × 5%)	**0.05%** (=0.26% x 5% + 0.82% x 5%)	**0.24%** (=1.18% x 5% + 3.60% x 5%)	**0.24%**
Estimated number of CKD cases attributable to Cd exposure in the Spanish female population aged 50-65 years*				
Spain	**2781**	**2300**	**10149**	**15230**

*: population numbers in 2009 (year the BIOAMBIENT_ES study was conducted) were retrieved from Eurostat.

These calculations show that for CKD (stages 3-5), 0.24% of the disease burden in Spanish women with age 50-65 years is attributed to Cd.

Similar calculations were done for France (ENNS), which shows that 0.06 % of CKD (stage 3-5) in women with age 60-74 years is attributed to Cd exposure (data not shown).

6.3.3.2 Bone effects

6.3.3.2.1 Based on the BIOAMBIENT_ES and ENNS results

Based on the distribution of Cd concentrations in the urinary samples collected in the BIOAMBIENT_ES and ENNS studies, the fractions of urinary samples (collected in women aged > 50 years) exceeding the threshold U-Cd value of 0.50 µg/g crea for bone effects is indicated in Table 12, as well as the fractions of samples with U-Cd in the range > 0.5-0.75 µg/g crea and ≥ 0.75 µg/g crea.

Table 12: Distribution of the U-Cd concentrations measured in women aged above 50 years from the BIOAMBIENT_ES and ENNS studies and percentage of exceedance of the U-Cd threshold value for bones effect (0.5 µg Cd/g crea).

Country	Age (years)	N	U-Cd concentrations (µg/g crea)								% > 0.5 µg/g crea	% > 0.5 - 0.75 µg/g crea	% ≥ 0.75 µg/g crea
			GM	95% CI GM	P10	P25	P50	P75	P90	P95			
BIOAMBIENT.ES (Spain)	**50-65**	119	0.42	0.34 - 0.52	-	0.29	0.46	0.69	1.27	1.82	**42.32%**	**16.35%**	**25.97%**
ENNS (France)	**60-74**	421	0.43	0.40 - 0.46	0.20	0.29	0.42	0.65	0.99	1.15	**40.08%**	**22.38%**	**17.69%**

From the **Spanish BIOAMBIENT.ES study**, 42.32% women aged 50-65 years are exceeding the U-Cd threshold for bone effects of 0.5 µg/g crea, from which **16.35% at range 0.5-0.75 µg/g crea** and **25.97% above 0.75 µg/g crea**.

From the **French ENNS study**, 40.08% women aged 60-74 years are exceeding the U-Cd threshold for bone effects of 0.5 µg Cd/g crea, from which **22.38% at range 0.5-0.75 µg/g crea** and **17.69% above 0.75 µg/g crea**.

Data on the prevalence of osteoporosis at total hip or spine in women aged above 50 years was retrieved from the literature (see Table 6): 30% and 32% in Spain and France, respectively.

Based on this country-specific prevalence and the OR for osteoporosis at hip or spine from the Engström et al. (2011), the RR was calculated according to Equation 1 (see section 6.3.1) for U-Cd levels 0.5-0.75 µg/g crea and ≥0.75 µg Cd/g crea (Table 13).

Table 13: Relative Risk determined for Spanish and French population based on BIOAMBIENT_ES and ENNS data, respectively.

U-Cd levels (µg/g crea)	OR for hip or spine osteoporosis in all women according to Engström (2011) (95% CI)	RR based on the BIOAMBIENT_ES data	RR based on the ENNS data
0.50 - 0.75	1.61 (1.20 - 2.16)	**RR** *Spain (0.5-0.75)* = 1.61 / ((1- 0.30) + (0.30*1.61) = **1.38**	**RR** *France (0.5-0.75)* = 1.61 / ((1- 0.32) + (0.32*1.61) = **1.37**
≥0.75	1.95 (1.30 - 2.93)	**RR** *Spain (≥0.75)* = 1.95 / ((1- 0.30) + (0.30*1.95) = **1.52**	**RR** *France (≥0.75)* = 1.95 / ((1- 0.32) + (0.32*1.95) = **1.50**

6.3.3.2.2 Attributable Fraction and Attributable Disease Burden calculations

The ADB was calculated according to the schedule in Figure 3 (section 6.3.1.) and results are presented in Table 14.

Table 14: AF and ADB calculations based on BIOAMBIENT_ES and ENNS HBM data

Study (country)	Number of women*	% > 0,5-0.75 µg Cd/g crea	AF 1	ADB 1 (cases)	% ≥ 0.75 µg Cd/g crea	AF 2	ADB 2 (cases)	Total ADB (ADB 1 + ADB 2)
BIOAMBIENT.ES (Spain)	**4253701** aged 50-64 y	**16.35%**	**0.056**	**71318**	**25.97%**	**0.119**	**151341**	**222659**
ENNS (France)	**4373494** aged 60-74 y	**22.38%**	**0.072**	**100951**	**17.69%**	**0.081**	**112824**	**213775**

**The numbers of women were retrieved from Eurostat[12] and are those for the years the HBM studies were conducted (2009 for BIOAMBIENT_ES and 2007 for ENNS).*

In Spain, in the group exposed to Cd levels between 0.5 and 0.75 µg Cd/g crea, 5.6% of the burden of hip or spine osteoporosis is attributed to Cd exposure, while in the higher exposed Cd group (>0.75 µg Cd/g crea) this is 11.9%. In France, this is respectively 7.2% in the group exposed to Cd levels between 0.5 and 0.75 µg Cd/g crea and 8.1% in the higher exposed group (>0.75 µg Cd/g crea).

In absolute terms, 222659 women aged 50-64 years in Spain suffer from osteoporosis at hip or spine due to Cd exposure. In France, these are 213775 women aged 60-74 years.

6.3.3.2.3 Based on the DEMOCOPHES results

Again, the life-time PBPK model was used to identify "alert" levels of U-Cd at each age, but leading this time to reach an U-Cd concentration of 0.5 µg/g crea at age 55-60 years (Annex 3), considering a constant dietary intake of Cd (here 0.36 µg/kg bw/day, by using the ANSES biokinetic PBPK model). The mean U-Cd "alert" levels for the age ranges 35-40 years and 41-45 based on these simulations are presented in Table 15.

Table 15: Mean U-Cd alert values according to age ranges, based on a constant dietary intake (here 0.36 µg Cd/kg bw/day)

Age range (years)	Estimated bodyweight (kg)	Mean alert U-Cd levels*
35-40	73	**0.25 µg/g crea**
41-45	73	**0.34 µg/g crea**

** if the U-Cd concentration is higher than the alert value at this age category, the threshold of 1 µg Cd/g crea will be exceeded at the age of 55-60 years.*

[12] https://ec.europa.eu/eurostat

The percentages of women exceeding the mean U-Cd levels for their age range are representing the fractions of women at risk of exceeding the U-Cd threshold of 0.5 µg/g crea at age 55-60 years, provided they have a Cd dietary intake equal to the EFSA's TDI of 0.36 µg Cd/kg bw/day, from their age on to 60 years (see Annex 4).

However, when considering the average intake of Cd for European adults of 1.7 µg/kg bw/week (middle bound mean Cd exposure in adults), i.e. 0.24 µg/kg bw/day calculated in 2012 by EFSA, then:

- among the women aged 35-40 years, only those having an U-Cd level above 0.35 µg Cd/g crea will exceed the threshold of 0.5 µg/g crea at age above 55-60 years (if they have an average intake of Cd of 0.24 µg/kg bw/day from age 37 on).

- among the women aged 41-45 years, only those having an U-Cd level above 0.41 µg Cd/g crea will exceed the threshold of 0.5 µg/g crea at age above 55-60 years (if they have an average intake of Cd of 0.24 µg/kg bw/day from age 43 on).

The fractions of women exceeding these U-Cd levels are presented in Table 16.

Table 16: Fractions of women exceeding a certain level of urinary Cd crea for their age range at which a dietary intake of 0.24 µg/kg bw/day (average intake for EU adults) will lead to an exceedance of the U-Cd threshold of 0.5 µg/g crea and put them at increased risk of suffering from osteoporosis (femoral neck, hip and spine according to Engström et al. (2011)) (0.35 µg Cd/g crea from 35-40 to 60 years; 0.41 µg Cd/g crea from >40-45 to 50-60 years).

Country	Age	N	%>LOQ	GM (95% CI)	SD	% > 0.35 µg/g crea for the 35-40 years age range and % > 0.41 µg/g crea for the > 40-45 years age range (bones effect)
Be	35-40	58	100.0%	0.182 (0.153-0.217)	0.142	16,1%
	>40-45	45	97.8%	0.187 (0.151-0.233)	0.137	7.70%
Czech	35-40	56	100.0%	0.220 (0.200-0.250)	0.110	16.2%
	>40-45	24	100.0%	0.230 (0.190-0.270)	0.100	6.10%
Denmark	35-40	55	90.9%	0.105 (0.860-0.129)	0.103	6.10%
	>40-45	74	91.9%	0.134 (0.111-0.162)	0.129	5.50%
Spain	35-40	49	98.0%	0.215 (0.173-0.267)	0.233	13.3%
	>40-45	57	98.2%	0.223 (0.186-0.269)	0.328	20.8%
Hungary	35-40	45	81.25%	0.160 (0.140-0.200)	0.140	15.4%
	>40-45	23	73.91%	0.130 (0.100-0.180)	0.110	4.50%
Ireland	35-40	41	95.1%	0.247 (0.199-0.306)	0.216	28.7%
	>40-45	45	100.0%	0.325 (0.268-0.394)	0.247	35.8%
Lux	35-40	34	100.0%	0.207 (0.170-0.252)	0.146	23.9%
	>40-45	19	100.0%	0.225 (0.187-0.27)	0.830	1.60%
Poland	35-40	44	100.0%	0.371 (0.311-0.442)	0.349	39,9%
	>40-45	24	100%	0.492 (0.353-0.685)	0.550	61.1%
Portugal	35-40	52	97.2%	0.156 (0.131-0.186)	0.118	10.7%
	>40-45	29	100.0%	0.225 (0.185-0.273)	0.130	16.3%
Romania	35-40	46	86.8%	0.170 (0.136-0.212)	0.169	20.9%
	>40-45	7	77.8%	0.148 (0.060-0.370)	0.300	46.8%
Sweden	35-40	44	100.0%	0.124 (0.106-0.144)	0.066	1.06%
	>40-45	33	100.0%	0.161 (0.133-0.195)	0.110	3.70%

Country	Age	N	%>LOQ	GM (95% CI)	SD	% > 0.35 µg/g crea for the 35-40 years age range and % > 0.41 µg/g crea for the > 40-45 years age range (bones effect)
Slovenia	35-40	48	100.0%	0.222 (0.191-0.258)	0.122	18.1%
	>40-45	46	93.5%	0.252 (0.206-0.307)	0.150	25.4%
Slovak Rep	35-40	51	100.0%	0.220 (0.195-0.25)	0.112	15.1%
	>40-45	29	100.0%	0.290 (0.250-0.342)	0.125	20.7%
Switzerland	35-40	38	91.8%	0.207 (0.183-0.233)	0.110	12.2%
	>40-45	38	91.8%	0.207 (0.183-0.233)	0.110	5.10%

Table 17 summarises the total number of women aged 35-40 and 41-45 years in 14 DEMOCOPHES participating countries in 2011, the calculated fractions of women exceeding the U-Cd level at which a mean dietary intake of Cd of 0.24 µg/kg bw/day from their age to age 60 years will put them at higher risk of suffering from hip or spine osteoporosis (i.e. the threshold of 0.5 µg Cd/g crea will be exceeded at age 55-60 years), as well as the AFs and ADBs for each country and finally the total number of hip and spine osteoporosis cases among women due to Cd exposure for the 14 DEMOCOPHES countries.

Table 17: Number of women aged 35-40 and 41-45 in DEMOCOPHES participating countries, country-specific AF and ADB, total number of hip or spine osteoporosis cases due to Cd exposure in 14 DEMOCOPHES countries.

Country	N (women at age 35-40)*	% > 0.35 µg/g crea (prevalence expo)	AF 1	ADB 1 (cases)	N (women at age 41-45)*	% > 0.41 µg/g crea (prevalence expo)	AF 2	ADB 2 (cases)	Total number of cases
Be	443119	16.1%	0.054	**7406**	391833	7.7%	0.026	**3211**	**10617**
Czech	502239	16.2%	0.054	**8424**	339519	6.1%	0.022	**2308**	**10732**
Denmark	231977	6.1%	0.021	**1527**	204398	5.5%	0.020	**1266**	**2793**
Spain	2336156	13.3%	0.045	**32561**	1849883	20.8%	0.072	**41053**	**73614**
Hungary	459853	15.4%	0.052	**7369**	335081	4.5%	0.016	**1709**	**9078**
Ireland	214073	28.7%	0.092	**6120**	158764	35.8%	0.117	**5761**	**11881**
Lux	24058	23.9%	0.078	**581**	20927	1.6%	0.006	**38**	**619**
Poland	1577188	39.9%	0.124	**60560**	1149548	61.1%	0.184	**65681**	**126241**
Portugal	503083	10.7%	0.036	**5686**	397708	16.3%	0.057	**7013**	**12699**
Romania	909029	20.9%	0.069	**19377**	806628	46.8%	0.148	**36895**	**56271**
Sweden	373948	1.10%	0.004	**433**	325986	3.7%	0.013	**1350**	**1784**
Slovenia	85203	18.1%	0.060	**1593**	75456	25.4%	0.086	**2009**	**3602**
Slovak Rep	243027	15.1%	0.051	**3822**	177632	20.7%	0.071	**3917**	**7740**
Switzerland	338961	12.2%	0.041	4350	321370	5.1%	0.019	**1849**	**6198**
Total number of hip or spine osteoporosis cases due to Cd exposure in 14 DEMOCOPHES countries									**174060**

**The numbers of women were retrieved from Eurostat for 2011, when the DEMOCOPHES study was conducted.*

The AF of women aged 35-40y varied in the EU between 0.4% and 12%. For women aged 41-45y this was between 1% and 15%. If the intake of Cd is set to a constant value over time of 0.24 µg Cd/kg bw/day, this fraction of osteoporosis will be due to Cd exposure at later age.

6.3.4 Uncertainties

Uncertainty in the estimation of the ADB covers different aspects. A difference is made between context uncertainty, model structure uncertainty and parameter and input data uncertainty (Knol et al., 2010). Context uncertainty refers to choices made about system boundaries and definitions used in the assessment. Model structure uncertainty refers to uncertainty about the causal structure of the modelled system, uncertainty within the chosen boundaries. Parameter and input data uncertainty refers to uncertainty about the variables and input data used.

Regarding context uncertainty, we only focussed on osteoporosis at hip or spine and CKD, giving rise to an underestimation of the real Cd burden. Mortality (Tellez-Plaza et al., 2012), breast cancer (Gallagher et al., 2010; Julin et al., 2012), liver inflammation (Hyder et al., 2013), neurocognitive outcomes (Ciesielski et al., 2013), depressive symptoms (Scinicariello and Buser, 2015), age-related macular degeneration (Wu et al., 2014), prediabetes (Wallia et al., 2014) and lung cancer (Adams et al., 2012) are examples of adverse effects suspected to be partly related to Cd exposure. They were however not taken up in this ADB calculation, the reasons being mostly that relating the incidence of these endpoints in the general population to the Cd dose (at low-levels) and establishing a link of causality is not sufficiently substantiated by the body of evidence of available studies.

Focussing on model structure uncertainty: this can relate to applicability of the dose-response curve, for instance the use of a threshold. For osteoporosis, a threshold value of 0.5 µg Cd/g crea was applied based on the set-up of the study of Engström (OR < 0.5 µg Cd/g crea versus OR ≥ 0.5 µg Cd/g crea). Although it is mentioned in the study of Engström that no threshold was found for a restricted cubic spline model in the risk of osteoporosis at the femoral neck or lumbar spine (Engström et al., 2011). The LOD in the study was equal to 0.002 µg Cd/l and the 5th percentile of all samples equal to 0.15 µg Cd/g crea.

According to Nordberg et al. (2018) it is difficult to estimate the lowest exposures given rise to bone effects. The authors say it is not certain that there is a causal relationship between low concentrations of urinary Cd and decreased bone mineral density; consequently it is according to Nordberg and colleagues not possible to establish a LOAEL/BMD for this effect.

For CKD we started from the association between GFR and urinary Cd observed by Akesson et al. (2005). From the shift in GFR due to Cd exposure and definition of CKD, the ADB was calculated. However, there are other epidemiological studies that do not support the association between Cd exposure and the progression of CKD (Byber et al. 2016). Inconsistencies could be explained by e.g. differences in study design, co-exposure to other stressors. But, all the epidemiological studies suggesting no association were done in study population with a median/mean U-Cd level lower than 0.5 µg Cd/g crea (Zang et al, 2019). An analysis with NHANES data showed that moderately high level of U-Cd (≥1 µg/g crea) was associated with higher incidence of albuminuria, a well-known biomarker of renal dysfunction (Ferraro et al., 2010). Results of linking Cd exposure to reduced GFR at doses lower than 1 µg Cd/g crea remains to be clarified. Some reviewers find the evidence for the relationship sufficient, whereas other state that it is limited. More research can reduce this model structure uncertainty as it is predominantly epistemic. For the calculation of CKD

related Cd exposure we started from 1 µg Cd/g crea. Parameter and input data uncertainty can be addressed in a next version of this work.

7 Discussion and conclusions

The present document assessed the risk associated to Cd exposure of EU population. According to the EFSA reports (EFSA 2009a; 2012) which assessed the risk for dietary Cd exposure in the EU, the calculated Cd weekly intake was close or even exceeded the established TWI (especially in some population subgroups, as e.g. vegetarians and children). Taking advantage of the available biomonitoring data, and intending to improve the performed risk assessment, the attributable disease burden due to Cd exposure was estimated.

A large part of the BoD is attributed to chemical exposure (Prüss-Ustün et al., 2017). Some first economic estimates reveal that chemical exposure also entails a cost for society that may exceed 10% of the global domestic product (Grandjean and Bellanger, 2017). These calculations are based upon limited information on actual human exposure and related health outcomes for only a few chemicals, meaning the real burden is expected to be larger. Associations taken into account in these kind of estimates are for example:

- Exposures to lead, organophosphates, brominated flame retardants, methylmercury and IQ loss;
- Exposure to phthalates and obesity, diabetes and infertility; and
- Exposure to air pollution and premature mortality

Risks associated with exposure to arsenic and Cd were only considered as occupational in the 2013 Global Burden of Disease report (Forouzanfar et al., 2015), although they also occur in the general environment. In this exercise, a first attempt was performed to estimate the disease burden attributed to Cd exposure in the general population. This was done based on HBM data reflecting the integrated exposure. Focus was on CKD and osteoporosis (at hip or spine). The endpoints, taking effect at later age, were chosen seeing the increased body of evidence published during the last years. This also corresponds with Cd being built up in the kidney over time and the induction of bone effects, related to renal processes. However, there are still controversies concerning the effects of Cd at low dose, in particular on bones as only few studies have reported such effects with a dose-response relationship. Moreover, to have more confidence in these estimations, a causal relationship should be demonstrated between exposure to Cd and the diseases. We should recognize that for the time being there is still some gaps concerning the mode of action explaining effects of Cd on human health.

For CKD, the disease stages 3-5 (GFR <60 ml/min/1.73m^2) were considered. At stage 3, most of the patients will not show symptoms but some may already have swelling of hands and feet, back pain, abnormal urine production. The threshold below which no adverse kidney effects due to Cd exposure take place, was set at 1 µg Cd/g crea (in accordance with the EFSA 2009 U-Cd critical value, the HBM-I value and HBM4EU HBM-GV). Data of NHANES campaigns did also show an increased risk for CKD due to Cd exposure above this value (Ferraro et al., 2010). The exposure response function selected for calculating the attributable CKD burden due to Cd exposure was based on the study of Akesson et al. (2005) who measured U-Cd and markers of renal injury in 816 Swedish women aged 53-64 years.

For osteoporosis, the exposure response relationship was based on findings in the Swedish Mammography cohort (Engström et al., 2011). The threshold below which no adverse bone effects due to Cd exposure take place, was set at 0.5 µg Cd/g crea. Also in the US, increased hip-BoneMineralDensity–defined osteoporosis was found in women with urinary concentrations exceeding 0.5 µg Cd/g crea (Gallagher et al., 2008). However, it is difficult to estimate the lowest exposures giving rise to bone effects. According to Nordberg et al. (2018), it is not possible to be certain that there is a causal relationship at urinary Cd <5 µg Cd/g crea. Therefore, our estimate should be interpreted with caution and should not be used by policymakers at this stage. On the other hand, the precautionary principle should not be disregarded. What we can say is that more prospective studies with strong assessment of osteoporosis and major confounders are needed.

Two individual publicly available HBM studies in the EU, report on Cd concentrations in urine at later age (>50 y): the Spanish BIOAMBIENT_ES and the French ENNS studies. Respectively 17.4% and 8.0% of the women, aged 50-65 in BIOAMBIENT_ES and aged 60-74 years in ENNS, had a urinary Cd concentration > 1 µg Cd/g crea which means that there is an increased risk for health effects, not meaning that health effects are already present. It is an indicator that there is a concern of the exposure with regard to health-based criteria and is signal for policy-makers. The ADB for CKD, based on the available 10% highest exposure data (> P90 value which is > 1 µg Cd/g crea) of the BIOAMBIENT_ES study, was in the age category 50-64 y, equal to 0.24%. Similar results for the ENNS study showed an ADB of 0.06%. For osteoporosis, in the BIOAMBIENT-ES study, in the group exposed to Cd levels between 0.5 and 0.75 µg Cd/g crea, 5.6% of the burden of hip or spine osteoporosis was attributed to Cd exposure, while in the higher exposed Cd group (>0.75 µg Cd/g crea) this was 11.9%. In France, the burden of hip or spine osteoporosis attributed to Cd exposure was respectively 7.2% in the group exposed to Cd levels between 0.5 and 0.75 µg Cd/g crea and 8.1% in the higher exposed group (>0.75 µg Cd/g crea).

This approach was completed by another one in order to have a more exhaustive estimation by taking exposure to Cd at younger age (<50y) and assuming a PBPK model for lifetime exposure (ANSES, in prep.). HBM data for Cd are available at European scale through the DEMOCOPHES project (age categories with boundaries: 35-40y and >40-45y). The DEMOCOPHES project included 1844 mothers from 17 European countries, but the study was not representative for the whole of Europe. Based on a PBPK model for lifetime exposure with constant dietary intake over time for all EU countries (0.72 µg Cd/kg bw/day), the critical U-Cd concentration at the age categories 35-40y and 40-45y was calculated. People who already exceed these values are expected, if there is no significant changes in their exposure to Cd, to exceed the critical 1 µg Cd/g crea concentration at the age of 55-60 (TDI EFSA (2009)). The calculations show that the countries Spain, Ireland, Poland and Romania in both age categories (35-40y and 41-45y) exceeded the age specific limit value with more than 5%. This means that the available P95 value of exposure divided by the age corrected HBM-GV (the RCR) is >1. A similar kind of calculation (critical age-specific U-Cd concentration for not exceeding 0.5 µg Cd/g crea at age 55-60) was done for hip or spine osteoporosis as endpoint. It was clear that 1% (Sweden) to 61% (Poland) of the DEMOCOPHES mothers (age categories 35-40 and 41-45 considered) would exceed 0.5 µg Cd/g crea at age 55-60. The AF for osteoporosis for these women varied in the EU between 0.4% (Sweden) and 18% (Poland). If the intake of Cd is set to a constant value over time of 0.24 µg Cd/kg bw/day, this fraction of osteoporosis will be due to Cd exposure at later age.

Different types of uncertainty are involved in this kind of calculation going from context uncertainty, model uncertainty and input data uncertainty. We only focussed on osteoporosis

and CKD. For other endpoints, the evidence for a causal effect is less clear, meaning the ABD may be underestimated (context uncertainty). For osteoporosis the threshold value under which no effects are observed (set here at 0.5 µg Cd/g crea) can be debated, which is model uncertainty. Also, the application of a PBPK model for lifetime exposure includes uncertainty. Indeed, variability related to the modification of body weight and creatinine excretion with age is included in the model, but e.g. metabolic differences between children and adults are not accounted for. Therefore, results may be overestimated. Input data uncertainty was not yet taken into account in this document.

We stress that the current calculation was an exercise to include HBM data in an environmental BoD calculation and that the output should not yet be used by policymakers. Despite important, these results should be cautiously interpreted due to the assumptions and uncertainties considered (as detailed in section 6.3.4).

Through the applied approaches, the present study focused mainly the methodology to consider the HBM data in the Cd risk assessment and in the estimation of the ADB associated to Cd exposure. Risk assessment of Cd exposure taking into consideration the biomonitoring data was successfully implemented in the two case-studies, suggesting that the applied methodology addresses the established objectives. Evidence of significant exceedance of the U-Cd threshold value of 1 µg/g crea for women at age around 60 years is observed from the Spanish BIOAMBIENT_ES and the French ENNS studies, also from most of the DEMOCOPHES countries (results based on PBPK modelling and dietary intake assumptions). These results lends further support to the EFSA conclusion that Cd exposure in the general population should be reduced. It even strengthen this recommendation, as when considering the aggregated exposure to Cd through available HBM results, the fraction of women exceeding the U-Cd threshold value for renal effects could reach up to 32% (DEMOCOPHES - Poland results).

Future efforts should be implemented to reduce the associated sources of uncertainty, especially to improve the strength of evidence of Cd dose-response curves. This is a crucial aspect to refine in the future the risk assessment using biomonitoring data.

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9 Annex

9.1 Annex 1: Slovenian case-study on the link between Cd soil contamination and human Cd dietary exposure

One of the policy question on Cd that is important to be covered under Task 5.3 relates to the link between Cd high soil contamination and human exposure via dietary sources. ANSES recently worked on a proposal for Cd maximum levels in fertilizing materials and culture media, in order to control the Cd levels in agricultural soils and subsequently the Cd contamination of plants consumed by humans. A mathematical predictive model was elaborated for estimating the evolution of contamination of Cd in plants intended for human consumption (based on the input of Cd in soils) over a 99 years projection time (due to the persistence behavior of Cd). The assessment focused more particularly on wheat and potatoes, two plants identified as the origin of major food contributors to the Cd exposure of French consumers together with the use of various scenarios of soils fertilization *via* fertilizers materials, resulting in a major supply of Cd in the food chain. The process consisted in two steps:

- Firstly, the transfer of Cd *via* fertilizers on agricultural soils to the plant production (potato and wheat grain) was modeled. This part of the model was built on the basis of a "mass-balance" approach, taking into account: 1) all the routes of Cd entry into the agricultural soil (fertilizing materials, atmospheric deposition, irrigation water); 2) routes of Cd release from the soil (food crops, leach); 3) variabilities and also 4) French specificities along this transfer. This first phase of the model makes it possible to study the Cd contamination of agricultural soils and of crops, as a function of Cd inputs via fertilizer materials and the agricultural practices over a projection time of 99 years.

- Secondly, the transfer of Cd from the plant to the consumers through diet was modeled, in order to estimate the impact on the consumer exposure. Simulations results of various fertilization scenarios were used as inputs in a model developed to evaluate the dietary exposure associated with the maximum levels of Cd in foodstuffs, to obtain the variations of Cd concentration (reduction or increase) in wheat and potatoes. The resulting variations allowed for estimating the Cd exposure impact on the consumers and identification of the adult and child consumer's mean chronic exposure (also the 95th percentile), as a function of the modelisation projection time (10, 20, 60, 99 years) and of the evolution of the Cd contamination in crops (wheat grain and potato) linked to fertilization scenarios. It allowed also for assessing possible exceedance of the Cd toxicological reference value (external for oral chronic exposure, but also internal as the U-Cd critical concentration).

The mathematical model developed to predict the temporal evolution of Cd concentrations according to different input fertilizer scenarios was parameterized to Slovenian specific data (e.g. soil specificities, Cd atmospheric and water concentrations, etc), allowing to further assess the impact of variations of Cd concentrations in agricultural soils on the dietary exposure of the Slovenian general population, which can be reflected by the human internal Cd concentration (blood or urine).

This model is now accessible via an R-shiny web application, under: https://shiny-public.anses.fr/cadmium_sl/.

Contributing partners to this work are from JSI (David Kocman, Janja Snoj Tratnik, Tine Bizjak) and from ANSES (Louis Trocellier, Amélie Crépet, Eva Ougier).

9.2 Annex 2: U-Cd alert levels according to age, for renal effects

Limit (median) values of U-Cd (µg/g crea) according to age to reach the threshold limit value of 1 U-Cd µg/g crea at age 55-60.

Time (year)	Body weight (kg)	U-Cd (ug/g crea)
1	8	0.03
2	12	0.06
3	16	0.08
4	20	0.09
5	24	0.10
6	27	0.10
7	31	0.11
8	34	0.12
9	37	0.12
10	40	0.13
11	42	0.14
12	45	0.14
13	47	0.15
14	50	0.16
15	52	0.16
16	54	0.17
17	56	0.18
18	58	0.19
19	59	0.19
20	61	0.20
21	62	0.21
22	64	0.22
23	65	0.23
24	66	0.24
25	67	0.26
26	68	0.27
27	69	0.28
28	69	0.30
29	70	0.31
30	71	0.33
31	71	0.35
32	71	0.37

Time (year)	Body weight (kg)	U-Cd (ug/g crea)
33	72	0.39
34	72	0.42
35	72	0.44
36	73	0.47
37	73	0.49
38	73	0.52
39	73	0.55
40	73	0.58
41	73	0.61
42	73	0.65
43	72	0.68
44	72	0.71
45	72	0.75
46	72	0.78
47	72	0.81
48	71	0.84
49	71	0.86
50	71	0.89
51	71	0.91
52	70	0.93
53	70	0.95
54	70	0.96
55	70	0.97
56	69	0.98
57	69	0.98
58	69	0.98
59	69	0.98
60	69	0.99
61	69	0.99
62	69	0.99

9.3 Annex 3: U-Cd alert levels according to age, for bone effects

Limit (median) values of U-Cd (µg/g crea) according to age to reach the threshold limit value of 0.5 U-Cd µg/g crea at age 55-60.

Time (year)	Body weight (kg)	U-Cd (ug/g crea)
1	8	0.01
2	12	0.03
3	16	0.04
4	20	0.04
5	24	0.05
6	27	0.06
7	31	0.06
8	34	0.06
9	37	0.07
10	40	0.07
11	42	0.07
12	45	0.08
13	47	0.08
14	50	0.09
15	52	0.09
16	54	0.09
17	56	0.10
18	58	0.10
19	59	0.11
20	61	0.11
21	62	0.12
22	64	0.12
23	65	0.13
24	66	0.14
25	67	0.14
26	68	0.15
27	69	0.16
28	69	0.16
29	70	0.17
30	71	0.18
31	71	0.19
32	71	0.20

Time (year)	Body weight (kg)	U-Cd (ug/g crea)
33	72	0.22
34	72	0.23
35	72	0.24
36	73	0.26
37	73	0.27
38	73	0.29
39	73	0.30
40	73	0.32
41	73	0.33
42	73	0.35
43	72	0.37
44	72	0.38
45	72	0.40
46	72	0.41
47	72	0.43
48	71	0.44
49	71	0.45
50	71	0.47
51	71	0.48
52	70	0.48
53	70	0.49
54	70	0.49
55	70	0.50
56	69	0.50
57	69	0.50
58	69	0.50
59	69	0.50
60	69	0.49
61	69	0.49
62	69	0.49

9.4 Annex 4: Exceedance of U-Cd alert levels for bone effects

Distribution of the U-Cd concentrations measured in women from the DEMOCOPHES studies (for 14 participating countries) and percentage of exceedance of the mean U-Cd alert level for bones effects calculated for each age range (35-40 years = 0.25 µg Cd/g crea; 41-45 years = 0.34 µg Cd/g crea), by a constant Cd dietary intake (0.36 µg/kg bw/day)

Country	Age (years)	N	U-Cd concentrations (µg Cd/g crea)								% > U-Cd alert level
			GM	95% CI GM	P10	P25	P50	P75	P90	P95	
Belgium	**35-40**	58	0.182	0.153 - 0.217	0.102	0.116	0.173	0.311	0.414	0.541	31.5%
	41-45	45	0.187	0.151-0.233	0.091	0.144	0.208	0.267	0.371	0.462	13.9%
Czech Rep	**35-40**	56	0.220	0.200 - 0.250	0.130	0.170	0.220	0.290	0.430	0.470	39.3%
	41-45	24	0.230	0.190-0.270	0.170	0.190	0.220	0.280	0.420	0.420	19.3%
Denmark	**35-40**	55	0.105	0.860 - 0.129	0.045	0.070	0.111	0.170	0.254	0.385	12.4%
	41-45	74	0.134	0.111-0.162	0.044	0.088	0.147	0.252	0.320	0.417	9.20%
Spain	**35-40**	49	0.215	0.173 - 0.267	0.093	0.136	0.192	0.389	0.580	0.638	41.5%
	41-45	57	0.223	0.186-0.269	0.090	0.138	0.215	0.321	0.575	0.748	28.7%
Hungary	**35-40**	45	0.160	0.140 - 0.200	0.060	0.120	0.160	0.270	0.310	0.440	39.0%
	41-45	23	0.130	0.100-0.180	0.050	0.110	0.140	0.190	0.260	0.440	10.8%
Ireland	**35-40**	41	0.247	0.199 - 0.306	0.089	0.153	0.279	0.405	0.559	0.658	49.2%
	41-45	45	0.325	0.268-0.394	0.116	0.172	0.341	0.490	0.715	0.933	47.2%
Lux	**35-40**	34	0.207	0.170 - 0.252	0.119	0.135	0.23	0.345	0.433	0.589	39.1%
	41-45	19	0.225	0.187-0.270	0.102	0.172	0.213	0.281	0.340	0.361	9.10%
Poland	**35-40**	44	0.371	0.311 - 0.442	0.125	0.202	0.409	0.620	0.927	1.057	71.4%
	41-45	24	0.492	0.353-0.685	0.182	0.345	0.468	0.947	1.343	1.479	71.3%
Portugal	**35-40**	52	0.156	0.131 - 0.186	0.071	0.111	0.161	0.225	0.361	0.440	23.4%
	41-45	29	0.225	0.185-0.273	0.094	0.158	0.261	0.308	0.439	0.545	30.4%
Romania	**35-40**	46	0.170	0.136 - 0.212	0.053	0.136	0.178	0.279	0.406	0.681	33.2%
	41-45*	7	0.148	0.060-0.370	0.017	0.063	0.241	0.375	0.955	0.955	56.3%
Sweden	**35-40**	44	0.124	0.106 - 0.144	0.069	0.088	0.132	0.177	0.234	0.246	6.00%
	41-45	33	0.161	0.133-0.195	0.086	0.118	0.168	0.208	0.288	0.411	11.4%
Slovenia	**35-40**	48	0.222	0.191 - 0.258	0.134	0.16	0.229	0.337	0.422	0.509	40.6%
	41-45	46	0.252	0.206-0.307	0.115	0.189	0.286	0.383	0.530	0.545	41.2%
Slovak Rep	**35-40**	51	0.220	0.195 - 0.250	0.116	0.158	0.225	0.311	0.382	0.454	38.8%
	41-45	29	0.290	0.250-0.342	0.144	0.234	0.295	0.436	0.511	0.514	42.9%
Switzerland	**35-40**	38	0.207	0.183 - 0.233	0.119	0.166	0.202	0.288	0.383	0.411	33.7%
	41-45	38	0.207	0.183-0.233	0.119	0.166	0.202	0.288	0.383	0.411	16.1%

*The number of individuals is limited.

Annex C: chromium(VI) risk assessment

D5.5 Substance-group specific risk assessment for Chromium(VI), occupational exposure

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1 Introduction

The research question is how HBM data could improve this risk assessment for occupational exposure to Cr(VI) compounds at surface treatment in maintenance of military equipment and at stainless steel welding. In stainless steel welding, co-exposure to another lung carcinogen, nickel, occurs.

This question is in line with the objectives of WP5 (to improve chemical risk assessment through the use of HBM data and to bring the results of HBM forward in terms of possible options for policy action and risk management).

In the Netherlands, an occupational risk assessment for surface treatment was required recently from RIVM by the Ministry of Defence in view of historic exposure of a total of 3000 workers involved in maintenance of military equipment at five NATO equipment storage sites (Prepositioned Organizational Material Storage sites, POMS) between 1984 and 2006. Adverse health effects such as lung cancer, nose and nasal sinus cancer, nasal septum ulcerations, chronic lung diseases, respiratory allergy (asthma and rhinitis) and allergic contact dermatitis were reported by workers which may be related to this exposure (RIVM, 2018; Palmen et al., 2018). At these sites, employees of the Ministry of Defence carried out maintenance activities on NATO equipment. The main source of hexavalent chromium [Cr(VI)] was the primer coating used to protect the equipment, and maintenance activities such as painting, blasting, sanding, grinding, welding and (dis)assembling could cause the release of this substance. Exposure information was very scarce for these workshops and only crude estimates were available resulting in a semi-quantitative risk assessment.

Workers are exposed to much higher levels of Cr(VI) compounds than consumers, since Cr(VI) compounds are no longer permitted in many consumer products and, if they occur, are present as impurities at low levels.

2 Methodology

The methodology used in this risk assessment included:

- Summary of the hazard assessment with an evaluation on dose-responses. Health hazards of Cr(VI) compounds have extensively been reviewed recently by the Agency for Toxic Substances and Disease Registry (ATSDR) 2012), the International Agency for Research on Cancer (IARC) 2012, the National Institute of Occupational Safety and Health (NIOSH) 2014, European Food Safety Authority (EFSA) (2014,2015)) and

the Health Council of the Netherlands (on reproduction, 2016 and on carcinogenicity, 2016). Their evaluations and conclusions were used as the starting point for this review. Additionally, a literature search[13] was conducted by the RIVM for publications on (additional) health hazards and recently published literature of Cr(VI) compounds not yet included in these evaluations. The RIVM search covered the period of January 2012 until June 2016, searching MEDLINE, EMBASE, Scopus, Toxicology Literature Online (TOXLINE) databases for studies about health hazards of Cr(VI) compounds. (Section 3). The final inclusion criteria of the full text screening were: 1) health effects of Cr(VI) compounds described, 2) Dutch or English language, 3) risk assessment of Cr(VI) compounds in animals or humans. This full text analysis resulted in n=49 human studies and n=24 animal studies used for the data-extraction.

- Summary of the exposure assessments done so far and an evaluation of the literature on correlations between biomarkers and external exposure. (Section 4)
- Risk characterization and uncertainty analysis (Section 5)
- Improving the risk assessment by biomonitoring (Section 6):
    - Evaluation of the available biomonitoring data
    - Risk assessment for the sample populations (Dutch military workers, Finnish surface treatment/welding workers)

All Cr(VI) compounds (chromates) combined will be considered. Paints usually contain chromates of Zn, Ba, Sr, Pb.

3 Summary of hazard characterisation

Occupational exposure to Cr(VI) compounds mainly occurs through the inhalation and dermal route including oral absorption due to hand to mouth behaviour. Palmen et al. (2018) categorized human diseases according to the likelihood of a causal link with exposure to Cr(VI) compounds based on existing knowledge from available studies in humans and animals, as 1) likely in humans, 2) possible in humans, 3) insufficient evidence in humans, and 4) unlikely in humans. Categorization was restricted to irreversible adverse health effects.

Exposure to Cr(VI) compounds is likely to cause lung cancer, nose and nasal sinus cancer, nasal septum ulcerations, chronic lung diseases, respiratory allergy (asthma and rhinitis) and allergic contact dermatitis in humans. Exposure to Cr(VI) compounds possibly causes stomach cancer in humans.

There is evidence from animal studies that Cr(VI) compounds can cause reproductive or developmental toxicity effects. However, there was insufficient human data available to draw any conclusion about the human relevance of these findings in animal studies. Therefore, it was concluded that there is insufficient evidence in humans that Cr(VI) compounds can cause reproductive or developmental toxicity effects.

It was considered likely that exposure to Cr(VI) compounds may induce allergic contact dermatitis, respiratory allergy and chronic lung diseases in humans.. Although animal studies give some indications of (intermediate) effects on the immune system following exposure to

[13] Keywords: (Chromium Compounds OR Chromium OR chrome$ or chromi$ or cr) adj ('6' or VI)) OR ((chrome$ or chromi$ or cr) adj hexaval$) OR (hexaval$ adj (chrome$ or chromi$ or cr))) AND (Occupational Diseases OR Occupational Exposure OR Chronic Disease OR Environmental Health OR Environmental Exposure OR Health OR Maximum Allowable Concentration OR Risk OR Risk Assessment OR Accidents OR Hazardous Substances OR diseases)

Cr(VI) compounds, no scientific evidence was available to relate these effects to any irreversible immune-mediated human diseases (other than allergic contact dermatitis, respiratory allergy and chronic lung diseases).

Lastly, based on the evidence available, it is unlikely that exposure to Cr(VI) compounds causes larynx cancer, intestinal cancer, dental effects and irreversible adverse health effects on the gastrointestinal tract, the haematological system, the liver, the kidneys, the neurological system and the cardiovascular system (Suh et al. 2019).

Dose-response assessment

The dose-response for lung cancer caused by exposure to Cr(VI) compounds was investigated in 5 epidemiological occupational studies of high quality, including the 'Baltimore-' or 'Painesville'-cohorts. Seidler et al. (2013) evaluated these data and concluded on a dose-effect relationship as follows:

$$RR = 1.75 \times C + 1$$

in which RR is the Relative Risk and C the cumulative exposure in mg/m^3-years. The Attributive Risk (AR) was calculated using the formula:

$$AR\ (\%) = 100 \times (RR - 1) / RR$$

It was not possible to derive dose-effect relationship for stomach cancer and nose and nasal sinus cancer based on human data. No thresholds could be established for allergic contact eczema, allergic asthma and rhinitis.

There were insufficient data to derive a dose-effect relation for chronic lung disease, although it is known that around 20 µg/m^3 symptoms start to develop (Palmen et al., 2018). A threshold for nasal septum ulcerations is difficult to establish considering additional exposure to Cr(VI) via hand-nose contact. Based on Lindberg and Hederstierna (1983) and Lucas and Kramkowski (1975) it was concluded that chronic exposure from 1 µg/m^3, in combination with dermal exposure, will result in an increased risk for this adverse effect.

A threshold for adverse effects on fertility and prenatal effects is based on inhalation studies with rats. A NOAEC of 200 µg/m^3 was derived (Glaser et al., 1984). This concentration was the only tested one and therefore the real NOAEC may be higher. Moreover, this NOAEC was based on a nearly continuous exposure frequency of 22 hr/day, 7 days/week (in total 154 hr/week) compared to the occupational exposure pattern of 8 hr/day and 5 days/week (in total 40 hr/week). The NOAEC of 200 µg/m^3 therefore can be considered a worst-case estimate in the Margin of Safety approach.

SCOEL (2017) concluded on risk levels for cancer due to exposure to Cr(VI) compounds. Excess risk benchmark values of 4/1000 and 4/100 000 workers were estimated to be related to exposure levels of 1 and 0.01 µg/m^3, respectively. These estimations were based on the dose-response function of Seidler et al. (2013) in humans, described above, and are similar to the exposure estimates which have recently been published by other organisations and researchers and are presented in an overview produced by the Dutch Expert Committee for Occupational Standards (Health Council of the Netherlands, 2016b).

In relation to the Cr(VI) substance authorisation process under REACH, the Risk assessment committee (RAC) of the European Chemicals Agency (ECHA) has performed dose-response assessment for Cr(VI) substances concerning carcinogenicity (RAC 2013) and reproductive toxicity (RAC 2015) arising from the Cr(VI) ion. For the lung cancer risk, like in the SCOEL assessment, an excess risk of 4/1000 in workers was calculated at 1 µg/m^3, based on the

same dose-response function (Seidler et al. 2013). For the particles that are cleared from the upper respiratory tract and swallowed, a cancer risk of the small intestine was assessed as 2/1000 in workers at 1 µg/kg/day (oral) by RAC. This risk estimate was based on the analysis by USEPA (2012), which used the NTP bioassay in rats and mice (NTP 2008) for the dose-response assessment. An oral cancer slope factor, representing the excess cancer risk at a dose of 1 mg/kg bw/day, was derived using benchmark dose modelling, and the slope value converted to human equivalents. For reproductive toxicity, reference DNELs were derived for workers as 43 µg/m^3 (inhalation, dermal) for fertility effects, and 85 µg/m^3 (inhalation) and 93 µg/m^3 (dermal) for developmental effects. The evaluation was based on deriving LOAEL values from *in vivo* studies as point of departure and applying route-to-route extrapolation. The LOAEL used for derivation of the reproductive toxicity DNEL was 5.2 mg Cr(VI)/kg bw/d, originating from a rat 6-day oral study (testicular toxicity). The LOAEL used for the derivation of the fertility DNEL was 7.9 mg Cr(VI)/kg bw/d, originating from a rat oral developmental toxicity study (GD 6-15, several foetal effects in the absence of significant maternal toxicity).

4 Summary of available exposure assessment

4.1 Cr(VI) and surface treatment of military equipment

The extent to which Ministry of Defence personnel at the five POMS sites came into contact with Cr(VI) compounds differed according to their positions (Palmen et al., 2018). Employees in technical maintenance positions were the most exposed to Cr(VI) compounds. This mainly took place during activities, such as sanding, carried out on equipment that had been treated with paint containing Cr(VI) compounds but also when coating equipment with paint containing Cr(VI) compounds.

The Cr(VI) compounds to which Ministry of Defence personnel were exposed in the period 1984-2006 can no longer be detected in their bodies, as, based on kinetic knowledge, Cr(VI) is converted to trivalent chromium [Cr(III)] in the body and subsequently excreted.

Four exposure groups were identified. These were, in order of highest to lowest exposure:

1) Specific functions for which direct inhalation exposure was possible as well as skin and oral exposure.
2) Functions for which it can be assumed that there was background exposure by inhalation. Indirect exposure of skin and oral intake was possible.
3) Functions for which incidental direct inhalation exposure was possible. Indirect inhalation and skin exposure as well as oral intake was also possible.
4) Functions with negligible exposure.

Semi-quantitative exposure assessments were performed for these exposure scenarios. For the first group, exposed before 1990, it appeared feasible to derive a worst case median daily average concentration per year of 20 µg Cr(VI)/m^3. This value has been used as reference point for inhalatory exposure for lung cancer and for reproductive effects. In more detail:

1) Direct contact with Cr(VI) during jobs such as: painting, blasting, sanding, grinding, welding and (dis)assembling. Functions were: technician, mechanic, welder, derust repair man, sprayer, blaster and cooperating foreman. Results of the exposure estimation:
   - Highest exposures of up to median daily average of 20 µg/m^3

- Exposure of cooperating foreman: 1984 up to 1990 10 µg/m^3, period 1990-1999 5 µg/m^3 and period after 1999 up to 2006 2.5 µg/m^3;
- Exposure of blaster: entire period 1 µg/m^3;
- Exposure sprayer: up to 1990 1 µg/m^3 and from 1990 0.2 µg/m^3;
- Exposure other functions: up to 1990: 20 µg/m^3, period 1990-1999 10 µg/m^3, period after 1999 5 µg/m^3;
- Exposure all functions:
    - Short-term, job-related exposures of high intensity possible
    - Direct and indirect skin exposure possible
    - Exposure via ingestion possible, via food and by hand-mouth contact.

2) Quality inspector is the worst case in this group: the worst case median daily average per year is lower than for group 1. Further quantification is not possible. Indirect skin exposure is possible as well as indirect oral exposure via hand-mouth contact. In this group, the installation technician can experience direct inhalation exposure and direct and indirect skin exposure; oral exposure via hand-mouth is possible albeit less frequent than for group 1; short-term high exposures can occur at similar intensity as for group 1 but at lower frequency.
3) Background exposure and indirect dermal and oral exposures at very low frequency cannot be excluded. Very low median daily average per year.
4) No exposure to Cr(VI) is assumed.

Outside the NL-study, monitoring data were found on surface treatment of Cr(VI)-containing paint layers NIOSH (2013):

- Removal of chromate containing paint by abrasive blasting (construction, spot abrasive blasting on steel bridge): exposure range in air (full shift, personal breathing zone) was 0.10–1.3 µg/m^3 (n=8, GM of 0.43 and GSD of 2.3). Exposures during blowdown and non-chromate repainting tasks was 0.077–0.29 µg/m^3 (n=7). Work inside containment area for environmental contaminants, natural ventilation only, low production job, spot blasting only.
- Spray application and re-sanding of chromate-containing paints (1-30%, painting in fully and partially enclosed paint booths, effectiveness judged as fair): exposure range in air (full shift, personal breathing zone) was 3.8–55 µg/m^3 (n=5, GM of 16 and GSD of 3.4). Exposure of painter's helpers in same work areas was 2.4–22 µg/m^3 (n=4).
- Spray application and some sanding of chromate-containing paints (1-30%, painting in fully enclosed paint booths, vacuum-attached disc sanders, effectiveness both judged as fair): exposure range in air (full shift, personal breathing zone) was 0.02–4.3 µg/m^3 (n=13, GM of 0.23 and GSD of 6.3). Exposure of assemblers using rotary-disc sanders was 0.27–2.1 µg/m^3 (n=4).

Commonly in workplaces, the chemical products used are mixtures of several substances. Additionally, in some workplaces, to obtain a final product with a specific quality and performance, several substances are used sequentially. For instance, for the first case, painting and welding are some of the examples and, for the second situation, plating a product can be mentioned. In both cases, exposure to Cr(VI) compounds occurs simultaneously with other substances that may have the same target organ. Therefore, additive effects can occur. For instance, in coatings applied in aircraft maintenance, it is common to have strontium chromate, crystalline silica and titanium dioxide in the same product. Meanwhile, in the case of the plaiting process for aircraft maintenance, also nickel is

used, and workers are exposed simultaneously to both substances. The welders can be exposed to the same kind of mixture since most of the welded material is similarly composed of both nickel and chromium. Likewise, surface treatment of coated materials will result in combined exposures to dust and the chemicals contained in it.

Recognizing the presence of these types of mixtures allows better understanding of the limitations of the risk assessment and Occupational Exposure Limits (OELs) based on single substances. It may also explain the cases where health effects are observed even when exposure values are below the OELs. This also brings higher uncertainty to the risk assessment process.

Conclusion:

A semi-quantitative exposure assessment could be performed for the first group of workers resulting in worst case median daily average concentrations per year.

The NIOSH data are difficult to compare but seem to be in the same order of magnitude for the same jobs (blasting, spraying in closed booths around 1 µg/m^3, range 0.02–4.3 µg/m^3).

4.2 Cr(VI) in REACH authorisation

Cr(VI) compounds (chromium trioxide, dichromium tris(chromate) and chromates) are authorised under the European regulation (EC 1907/2006) concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). According to REACH legislation, all companies using Cr(VI) compounds have to apply for authorisation for their uses. More than 100 authorisations for different uses of chromates have already been requested, some of these covering hundreds of workers, which means that potentially thousands of workers are exposed to Cr(VI) compounds in these activities (https://echa.europa.eu/applications-for-authorisation-previous-consultations).

In a survey performed to the REACH authorisations processes available in November/December 2018 (accessible at https://echa.europa.eu/applications-for-authorisation-previous-consultations) related with the use of chromates for surface treatment (essentially by plating, sanding and spraying) and linked with several substances in different concentrations. It was possible to observe that workers exposure estimation obtained was always below 2 µg/m^3(Annex 1) This value resulted, in most of the cases, from modelling even when measured data was available. In the cases that estimation was obtained through measured data, further adjustments were done for the use of respiratory protection devices and for the frequency of the task being considered. Less common were the authorization processes that have biomonitoring data available since in many cases the applicants claimed confidentiality reasons to not present the data, even when the companies have biomonitoring programs in place.

5 Risk characterisation and uncertainty analysis

5.1 Quantitative assessment

Lung cancer

Group 1, other functions, worker with 21 years of exposure (1984–2006): maximum exposure can be calculated to be 6 [yr] x 20 [µg/m^3] +10 [yr] x 10 [µg/m^3] + 5 [yr] x 5 [µg/m^3] = 245 µg/m^3 yr. The Relative Risk is 1 + 1.75 x 0.245 = 1.43. The indicative Attributable Risk [chance that lung cancer is caused by exposure to Cr(VI)] is 0.43/1.43=0.30 or 30%. See Table 1 for ARs of all functions in Group 1.

Table 1: Indicative AR for exposure Group 1.

Function	Exposure of 1 yr	Exposure of 5 yr	Exposure of 10 yr	Exposure of 21 yr
Technician, assembler, welder, derust repairman (other functions)	3.4%	15%	22%	30%*
Cooperating foreman	1.7%	8.0%	12%	18%
Sprayer	0.17%	0.87%	1.2%	1.6%
Blaster	0.17%	0.87%	1.7%	3.5%

* See example calculation

Calculation of the absolute risk for this worker:

Risk to be diagnosed with cancer during a lifetime (0-85 yr) in The Netherlands is 7.7% for men (IKNL data for 2005-2009 (http://www.cijfersoverkanker.nl/selecties/Dataset_2/img5a6f065ee0446). Since RR = 1.43, this means that the risk for (death by) lung cancer is increased from 7.7% to 11.0% (1.43 x 7.7).

Fertility and developmental effects

A Margin of Safety (MoS) of 100 was calculated between the NOAEC of 200 mg/m^3 in rats, recalculated to a daily inhalatory dose of 0.3 mg/kg bw (Health Council of The Netherlands, 2016b), and the worst case median daily average per year of 20 µg/m^3 (Group 1). This exposure was recalculated to a dose of 0.003 mg/kg bw (body weight of 70 kg, inhalation volume of 10 m^3 during an 8-hr shift. The MoS of 100 was considered sufficiently large to account for uncertainties due to inter-and intraspecies differences (assuming a default margin of 10x10), also in view of the almost continuous exposure of the experimental animals and the use of a NOAEL instead of a LOAEL. The contribution of dermal exposure of workers was considered small in view of low dermal absorption. Oral exposure via hand-mouth contact was not quantified.

Other endpoints

For other endpoints and for other Exposure Groups no quantitative exposure and risk estimates could be produced in view of lack of exposure data.

5.2 Qualitative assessment

In the absence of (semi-)quantitative exposure data, a qualitative estimation at exposure group level was made of the likelihood that the diseases with a causal link to Cr(VI) compounds are caused by exposure to Cr(VI) compounds at the POMS-sites. This risk is strongly related to the nature, intensity, length and frequency of exposure. See Table 2

Table 2: The likelihood that the diseases with a causal link to Cr(VI) compounds are caused by exposure to Cr(VI) compounds at the POMS-sites (Palmen et al., 2018)

Disease \ Function	1 Functions with possible direct exposure	2 Functions for which background exposure can be assumed	3 Functions with possible incidental exposure	4 Functions with negligible exposure
Lung cancer	+	+	+	n.a
Nose and nasal sinus cancer	+	+	+	n.a
Cr(VI)-related allergic contact eczema	+	+	+	n.a.
Cr(VI)-related allergic asthma and rhinitis	+	+	?	n.a.
Chronic lung diseases	+	-	-	n.a.
Perforation of nasal septum by Cr(VI) ulcerations	+	+	-	n.a.
Stomach cancer[14]	+	+	+	n.a.
Effects on fertility	-	-	-	n.a.
Developmental effects	-	-	-	n.a.

+ It is possible that this disease is caused by exposure to Cr(VI) at the POMS-sites.
? It is not clear whether this disease is caused by exposure to Cr(VI) at the POMS-sites
- It is unlikely that this disease is caused by exposure to Cr(VI) at the POMS-sites
n.a. Not applicable

5.3 Uncertainties

Exposure assessment: Exposure information was very scarce for the NL-workshops investigated and only crude estimates were available resulting in a semi-quantitative risk assessment.

Risk assessment: in view of the limitations of the exposure assessment and lack of dose-response information for effects other than lung cancer, only a qualitative estimation at exposure group level was made of the likelihood that the diseases with a causal link to Cr(VI) care caused by exposure to Cr(VI) compounds.

[14] There is limited evidence that stomach cancer can be caused by exposure to Cr(VI)

6 Improving the RA by biomonitoring

6.1 Evaluation of the available biomonitoring data

In the current risk assessment exercise, human biomonitoring data from the Finnish Institute of Occupational Health (FIOH) was used. The FIOH biomonitoring database consist of all the biomonitoring samples sent to the Institute for monitoring chemical exposure by the occupational health care units of the work places. Data for chromium exposure was available from the 1980s until 2016. For the comparison with the Dutch risk assessment, the data from years 1980-1999 was used (Table 3). The chromium analyses were at that time performed using EAAS, whereas later, urinary chromium was analysed using ICP-MS. During the whole study period, the laboratory has taken part in external and internal quality programmes, and has been accredited by the Finnish Accreditation Service (Finas T013) since 1990's. All the results are standardised similarly, and the results covering the whole study period are comparable.

There were more than 42000 urinary chromium samples. Only from a part of samples it was possible to identify the worker's job title and / or the work place. All samples were classified according to the Standard Industrial Classification TOL 2008 (SIC). The results of urinary chromium analysis were divided to the groups based on work task, SIC and exposure to different chromium compounds. The group of platers consists of electroplating process workers and of those whose work place was classified to 'Treatment and coating of metals', excluding those workers whose job title was grinder, welder etc. worker not exposed to Cr(VI) compounds.

A great part of sprayers worked in car repairing workshops, but also painters in different industrial areas were included. The sandblasting and other mechanical surface treatments group was collected according to job titles.

To "mechanics", all those workers were collected who had mechanic or installer in the job title. Maintenance workers were divided in two groups: mechanical maintenance workers and the repairs with chemicals etc. as the exposure to Cr(VI) compounds could be different. The group welders include all workers using different welding techniques as well metal sheet workers. Moulder, founder and all those work task from casting process who exposed to casting fumes were collected to the group "casting".

The FIOH HBM data (p95) was converted into corresponding air levels by employing previously published conversion equations for the calculations (Table 3). Several studies have described such correlations between urinary concentrations of total soluble chromium and Cr(VI) air concentrations. In a study of Pierre et al (2008), it was concluded that the level of soluble chromium in air of 6 µg/m^3 corresponded with urinary total chromium level of 0.29 µmol/l, although the correlation equation was not included in the publication. This is in accordance with an older study of surface treatment by Lindberg & Vesterberg (1983), in which air Cr(VI) level of 2 µg/m^3 corresponded to total urinary level of 0.10 µmol/l. Furthermore, a study by Chen et al. (2002) concluded on correlations in the same range, albeit slightly higher.

Two equations were used for the calculations, one published by Lindberg and Vesterberg (1983) and another by Chen et al. (2002). Both were based on measurements for chromium plating work. The equation of Lindberg and Vesterberg for chromium plating is:

$$y\ [Cr_{air}]\ (\mu g/m^3) = 0.43 + 0.013 \times [Cr_{urine}]\ (nmol/l),\ (r=0.71).$$

This equation is based on atmospheric samples taken over the full duration of a shift (day not specified) and sampling of urine at the end of shifts on the 2nd working day. The LOD for Cr(VI) in air was 0.2 µg/m^3 and for chromium (total) in urine 0.25 µg/l. Personal protective equipment was not mentioned. The sample size was 57. The equation of Chen et al. for hard chromium plating is:

$$y\ [Cr_{air}]\ (\mu g/m^3) = [[Cr_{urine}]\ (\mu g/g\ Cr) + 0.33\ ] / 1.86,\ (r = 0.81).$$

For this correlation equation, atmospheric samples were taken over the whole working day (7 h), and urine samples at end of week and end of shift. The LOD for atmospheric Cr(VI) was 2 ng/m^3, and for the urinary chromium the LOD was 0.2 ng/l. Personal protective clothing was worn, and the sample size was 30.

Other correlations have been published but were considered less relevant because of either low sample sizes, poor correlation coefficients, considerably higher measurement levels compared with the FIOH data, or unclear or inapplicable exposure scenarios. In some instances, neither the conversion equation nor data for deriving it was available.

Table 3: Finnish biomonitoring data (p95) from different surface treatment activities for soluble chromium in urine. The measured urine concentrations have been converted to air concentrations using published conversion equations, based on chromium plating activities.

Job title	Years	n	µmol/l	µg/l	µg/g of creatinine [a]	Air conc. (µg/m3) (Lindberg & Vesterberg, 1983)	Air conc. (µg/m3) (Chen et al 2002
Mechanics	1980-1989	94	0.36	18.72	16.55	5.1	9.1
	1990-1999	757	0.20	10.40	9.20	3.0	5.1
Sandblasting and other mechanical surface treatments	1980-2016	30	0.51	26.52	23.45	7.1	12.8
Welding	1980-1989	3232	0.77	40.04	35.40	10.4	19.2
	1990-1999	6806	0.40	20.80	18.39	5.6	10.1
Maintenance	1980-1989	36	0.81	42.15	37.27	11.0	20.2
	1990-1999	91	0.11	5.72	5.06	1.9	2.9
Mechanical maintenance	1980-1989	39	0.12	6.36	5.63	2.0	3.2
	1990-1999	495	0.17	8.84	7.82	2.6	4.4
Plating	1980-1989	771	0.46	23.71	20.97	6.4	11.4
	1990-1999	857	0.33	17.16	15.17	4.7	8.3
Spraying	1980-1989	213	0.17	8.66	7.66	2.6	4.3
	1990-1999	254	0.19	9.88	8.74	2.9	4.9
Casting	1980-1989	15	0.86	44.72	39.54	11.6	21.4
	1990-1999	107	0.51	26.52	23.45	7.1	12.8

[a] From µg/l to µg/g approximation: 1 l of urine contains 1 g creatinine

These exposure and risk levels presented in table 3, do not necessarily reflect the current exposure. For example in plating, the average levels have been more than halved from the levels seen pre-2000 (see Table 4). The current exposure levels can be compared to the exposure estimates made in REACH authorizations. As it can be seen, biomonitoring data from plating activities show slightly higher levels of exposure than the estimated average exposure level of 2 µg/m3 used in many REACH authorization applications. This may, however, be partly explained by the exposure via hands (hand-to-mouth behaviour), which has been in many studies shown to contribute significantly to total systemic exposure in metal sector.

Table 4: Finnish biomonitoring data (p95) on plating activities from 2010-2016. The measured urine concentrations have been converted to air concentrations using published conversion equations, based on chromium plating activities.

Job title	Years	n	µmol/l	µg/l	µg/g of creatinine [a]	Air conc. (µg/m3) (Lindberg & Vesterberg, 1983)	Air conc. (µg/m3) (Chen et al 2002
Plating	2010-2016	3631	0.12	6.33	5.60	2.0	3.2

[a] From µg/l to µg/g approximation: 1 l of urine contains 1 g creatinine

Uncertainty related to converting HBM data into estimated air concentrations using such equations can be caused by:

- Small sample sizes
- Conversion equations may be based on higher exposure levels than those measured nowadays, and extrapolation to lower exposures is needed. Especially the older equations can be based on considerably higher exposure levels than the HBM data.
- Lack of reliable, task-specific conversion equations. The conversion equations used here are derived from measurements regarding chromium plating work, which might, for instance, overestimate exposures related to welding (see further below).
- The assumption that total chromium in urine is exclusively related to inhalatory exposure to Cr(VI) compounds in air. This is, however, not always true. Especially in welding some of the urinary chromium may represent Cr(III) leading to an overestimation of the risk, which may result in overestimation of the Cr(VI) exposure and lung cancer risk.
- Regarding applicable conversion equations for welding, an additional uncertainty is related to the variation in the used welding techniques. They have also evolved during the ~40-year measurement period.
- Regarding specifically the use of the Chen et al. (2002) equation, an additional source of uncertainty is that the equation assumes creatinine corrected urine concentrations, for which an additional conversion had to be made for the FIOH data.
- Dermal and oral exposure may have occurred. Part of the urinary total chromium is in many cases derived from hands-to-mouth exposure. This is not usually considered to contribute to lung cancer risk, but may contribute to stomach cancer which has been demonstrated in animals after oral exposure. However, when converting urinary total chromium levels to air levels and calculating lung cancer risk based on that data, overestimation of the overall cancer risk is likely to occur because the lung cancer occurs at much lower doses as stomach cancers due to oral exposure.

6.2 Risk assessment for the sample populations

6.2.1 Finnish surface treatment/welding workers

The estimated increased lung cancer risks for different tasks were calculated based on the FIOH biomonitoring data from years 1980-1999 (Table 5). The cumulative exposure levels and risk calculations were performed as described in Section 3 and exemplified in Section 6.2.2.

Table 5: Estimated lung cancer risks related to (assumed) Cr(VI) exposure based on the FIOH HBM data. The exposure period is 20 years in 1980–1999, which roughly corresponds with the exposure period in the Dutch risk assessment (1984–2006).

Job title	20-year cumulative exposure level (1980–1999; mg/m^3 year)		RR		AR (%)	
	a	b	a	b	a	b
Mechanics	0.08	0.14	1.14	1.25	12.5	19.9
Sandblasting and other mechanical surface treatments	[c] 0.15	[c] 0.26	[c] 1.26	[c] 1.45	[c] 19.8	[c] 30.9
Welding	0.16	0.29	1.28	1.51	22.0	33.9
Maintenance	0.13	0.23	1.22	1.40	18.3	28.8
Mechanical maintenance	0.05	0.08	1.08	1.13	7.5	11.7
Plating	0.11	0.20	1.19	1.35	16.3	25.7
Spraying	0.06	0.09	1.10	1.16	8.8	13.8
Casting	0.19	0.34	1.33	1.60	24.6	37.5

The calculations are based on air concentration approximations based on equations by

[a] Lindberg and Vesterberg (1983) and

[b] Chen et al (2002).

[c] An estimate, calculated based on the measured p95 for a ~40-year period (1980–2016), due to small sample sizes.

6.2.2 Changes in the risk assessment for Dutch Defence workers based on the biomonitoring results

The tasks of 'sandblasting and other mechanical maintenance functions' in Table 3 seem to be closest to the tasks of Group 1 in the Defence cohort. The average P95 of exposures, based on biomonitoring over the years 1980–2016 is 7.1 or 12.8 µg/m^3. This is in the range of the worst case median daily average exposures estimated for Group1 (5-20 µg/m^3). It can be concluded that this will not significantly change the results of the quantitative lung cancer risk assessment, but rather confirm it. The maximum exposure (based on Lindberg & Vesterberg), can be recalculated as 6 [yr] x 7.1 [µg/m3] +10 [yr] x 7.1 [µg/m3] + 5 [yr] x 7.1 [µg/m^3] = 149 µg/m^3.year. The Relative Risk would be 1 + 1.75 x 0.149 =1.26 (as compared to 1.43).

For fertility and developmental effects, the Margin of Safety (MoS) for Group 1 can be recalculated to 282 (as compared to 100) between the NOAEC of 200 mg/m^3, recalculated to a daily inhalatory dose of 0.3 mg/kg bw, and the average P95 of 7.1 µg/m^3. This exposure was recalculated to a dose of 0.001 mg/kg bw (body weight of 70 kg, inhalation volume of 10 m^3 during an 8hr shift. This recalculated MoS is larger than the previously calculated MoS and obviously sufficient. It is noted that in the earlier assessment, MoS was based on the worst case estimate of the median daily average as opposed to the average P95 of exposures in Table 3.

Several uncertainties make small differences in risk estimates insignificant:

- The comparison of tasks between both studies is difficult due to lack of details on job descriptions and on exposure levels over time, duration, frequency.
- The uncertainty in the biomonitoring data such as the 1) assumption that all chromium in urine is from exposure to Cr(VI) compounds, 2) the assumption that all chromium in urine is from inhalatory exposure, ignoring oral and dermal exposure, and 3) the uncertainty in the conversion factor.
- Moreover, the number of monitoring samples is very small for this group (30), even with all of the 40-year measurement data combined. In addition, the combined P95 includes measurements also from 2000-2016, for which in several of the other groups, the measured Cr-urine concentrations are much lower than in 1980-1999 (data not shown). This could, at least in theory, lead to underestimation of the exposure in the 80s and 90s.
- The uncertainties in the semi-quantitative, historic estimation of the exposure of the Defence cohort (see section 4).

It is also noted that current, lowest biological guideline or limit values in urine are exceeded for this cohort before the year 2000: ANSES established an 8-h OEL for Cr(VI) exposure of 1 µg/m^3 in the chrome-plating sector (ANSES 2017) based on a U-Cr BLV by ANSES of 2.5 µg/l (1.8 µg/g creatinine). In Finland, the current biological limit value for U-Cr is 20 µg/l corresponding to the current OEL of 5 µg/m3 as 8 h TWA. Before the year 2000, the limit values for Cr(VI) have been higher. E.g. Lauwerys and Hoet (2001) lists the following biological limit values for U-Cr:

ACGIH-BEI: 10 µg/g creatinine (increase during shift) and 20 µg/g creatinine (end of shift), corresponding to a TLV-TWA of 50 µg/m^3.

DFG 20 µg/l (end of shift) at an exposure of 50 µg/m^3 Cr(III).

Improvement by biomonitoring?

The biomonitoring results considered to be most related to the Defence workers cohort show, in spite of a number of uncertainties, that the lung cancer risk is not underestimated in the semi-quantitative risk assessment.

6.2.3 Changes for REACH authorisation?

Anonymised biomonitoring data can be used to quantitatively estimate internal dose or absorbed dose from all exposure routes such as dermal and hand-mouth route, besides inhalation. Particularly in the occupational settings considered in the survey performed for REACH authorisations processes (surface treatment) the hand-mouth route can be an appreciable exposure route when compared with the airborne inhaled dose, particularly when respiratory protection is used and workplace surfaces are contaminated with the substance. Moreover, the use of respiratory protection by workers is also difficult to take on board when

only using air monitoring to assess workers exposure. Therefore, biomonitoring data, even with some limitations in case of urine data (lack of specificity for Cr(VI)) can complement other exposure data (e.g. air concentrations measurement). In addition to presenting the magnitude of exposure, comparison of HBM data to air monitoring data can also help to identify the most relevant exposure route (inhalation or skin contact). This is of most importance for the selection of suitable and efficient risk management measures that should be dedicated and oriented to eliminate and/or control exposure. These aspects were probably the basis for some of the authorizations granted, which recommended the need to present anonymized biomonitoring data with sufficient contextual data in the future review reports.

7 Discussion and conclusions

This risk assessment was made to exemplify how HBM data could support risk assessment based on external exposure data. Maintenance of military equipment in The Netherlands was used as an illustrative scenario, which shows an increased health risk caused by exposure to Cr(VI) compounds. This risk assessment was supported by the biomonitoring data from Finland, despite of uncertainties especially with regard to the assignment of job titles and to the speciation of chromium exposure.

Based on the (semi)-qualitative assessment, the Dutch assessment (Palmen et al., 2018) concludes that it is likely that the diseases of Defence workers involved in different jobs involving Cr(VI)compounds may be caused by exposure to Cr(VI)compounds over the years 1980-2006. These diseases were lung cancer, nose and nasal sinus cancer, Cr(VI)-related allergic contact eczema, Cr(VI)-related allergic asthma and rhinitis, chronic lung diseases, perforation of nasal septum by Cr(VI) ulcerations and stomach cancer. The risk was strongly related to the nature, intensity, length and frequency of exposure.

In the current study a semi-quantitative risk estimate was made for lung cancer and shows an increased risk. Based on the semi-quantitative risk assessment the risk for lung cancer was increased from 7.7% to 11.0%. This was confirmed by historic biomonitoring data from Finnish workers. This analysis does not reflect on current exposure levels and therefore prompts further studies into current exposure scenarios for a large variety of jobs at a much broader geographical scale. Such studies are being performed within the scope of HBM4EU under WP8 and will make available a big data set of Cr in urine, RBC and plasma in workers performing several tasks, in diverse countries. Based on this new data, the risk assessment can be updated to the current situation by applying the methodology presented here. This new study under WP8 will also reply the policy questions on Cr(VI, which are:

1. What are the current exposure levels to Cr(VI) at workplaces in Europe?
2. What is the impact of authorisation and established limits of chromates on exposure levels (e.g. in surface treatment activities or in market products)?
3. What is the current exposure of the EU population to Cr(VI)?
4. Does exposure differ between countries? Why?

It should be noted that based on urinary chromium data, it is currently not possible to estimate the exposure of general population to Cr(VI) (policy question 3). This is because of the high intake of Cr(III) from different sources, and high urinary background Cr levels caused by Cr(III) intake. RBC-Cr analysis may, however, bring some information on this. This will be seen after completion of chromate study under WP8.

7.1 Future prospects and recommendations for the regulatory risk assessment

It is recommended to generate more occupational biomonitoring data in the EU in order to estimate the current exposure and risks caused by Cr(VI) in Europe. It is emphasized that Cr studies are underway in HBM4EU (WP 8.5), based on deliverable AD 8.2 Research plan for chromates study under HBM4EU.

When compared to U-Cr, the more specific biomarker for soluble Cr(VI) could be chromium in red blood cells (Lauwerys and Hoet, 2001). The usefulness of this biomarker in occupational biomonitoring (and also to identify background Cr(VI) levels of general population) is currently tested in WP8. In the body, Cr(VI) is reduced to Cr(III) and binds to haemoglobin, while Cr(III) cannot pass biological membranes, and therefore will not enter red blood cells. The concentration of chromium in red blood cells (analysed as total chromium as representing previously entered Cr(VI) only) will then reflect the exposure intensity to Cr(VI) via all routes during the lifetime of red blood cells. The risk assessment of Cr(VI) compounds can be improved with such data, provided appropriate exposure scenarios can be identified. However, biomonitoring is not indicated as a stand-alone exposure assessment tool, as it cannot make a distinction between oral, dermal and inhalatory exposure. This distinction is relevant for determining the risk of different types of cancer (lung, stomach). Therefore, environmental monitoring (air and/or dermal) will most likely be important to perform as well to have complementary data, and to identify the most relevant exposure route.

For occupational HBM, exhaled breath condensate (EBC) represents a new type of matrix and could be useful when there is a need to evaluate specifically lung cancer risk caused by inhalation exposure.

In the REACH authorization applications, biomonitoring data was often not available. In most cases, the applicants claimed confidentiality reasons for not presenting the data, even when the companies have biomonitoring programs in place. In the future, requiring anonymized HBM data in the authorization applications would be advisable, particularly when dermal and hand-mouth exposure routes can be a reality.

8 References

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Annex I: Summary of modelled and measured exposure results from REACH Cr(VI) autorisation applications (available in November 2018).

Authori-zation ID	Substance	% of Cr(VI)	Use (Spraying/platting/dyes)	WCS (chosen the ones of interest: *worst case scenario*)	RMM	Exposure estimation Air monitoring µg Cr(VI)/m^3	Exposure estimation Biomonitoring Chromium µg /L, creatinine corrected
0117-01	Strontium chromate	5-10%	Use of strontium chromate in primers applied by aerospace and defence companies and their associated supply chains	PROC 21,24: Sanding of large surfaces containing Cr(VI) in large work areas including cleaning	General ventilation: Wetting at the point of release / on-tool extraction / vacuum cleaning (90% reduction) RPE	1.2 Modelled ART 1.5 RPE adjusted	
0046-01	Strontium chromate	5-10%	Formulation of Mixtures	PROC 7: Surface treatment by spraying (large sized parts) in a purpose-designed room	Downward laminar flow booth RPE	0.26 Exposure value corrected for RPE TWA-8hrs [µg Cr(VI)/m3] (90th percentile)	
0046-02	Strontium chromate	< 10%	Application of paints, primers and specialty coatings containing Strontium Chromate in the construction of aerospace and aeronautical parts.	PROC 7: Surface treatment by spraying (large sized parts) in a purpose-designed room	Downward laminar flow booth RPE	0.83 Modelled ART 1.5 WCS	
0105-01	Sodium dichromate	61%	Repackaging of Sodium Dichromate to be supplied as a mordant in the dyeing of wool as sliver and/or yarn with dark colours in industrial settings	PROC 1,3,15,19:Use of chromium (VI) as a mordant in dyeing process	General ventilation and PPE	< 0.4 Measured data	<0.01 - 0.85
0072-06	Sodium dichromate	10- 50%	Industrial use of a mixture containing hexavalent chromium compounds for the conversion of cadmium coated circular and rectangular connectors	PROC 13: Manual dipping in rinsing baths	General ventilation : 3ACH Local exhaust ventilation (LEV), fixed capturing hood	0.0106 Modelled ART 1.5 Corrected for duration, RPE & frequency	
0043-03	Sodium dichromate	10- 50%	Use of Sodium dichromate for the electrolytic passivation of tin plated steel for the packaging industry	(PROC 2, 13, automatic or manual process - Passivation of tinplated steel (ETP) - by dipping/ immersion	Basic general ventilation (1-3 ACH)	1.26 Measured data	
0113-01	Sodium dichromate	Aqueous solution (46% water)	Dyeing of textile materials of wool is performed with mordant dyes with the use of sodium dichromate.	PROC 13 Use as mordant in wool dyeing	Mechanical ventilation and PPE (RPE)	0.428 Measured data	Biomonitoring data available
0072-07	Sodium dichromate	< 10%	Industrial use of a mixture containing hexavalent chromium compounds in conversion coating and passivation of circular and rectangular connectors	PROC 13: Manual dipping in Cr(VI) bath	General ventilation : 3ACH Local exhaust ventilation (LEV), fixed capturing hood Size of the work area ≥ 3000m^3	1.77×10^{-2} Modelled ART 1.5 Corrected for duration, RPE & frequency	
0063-02	Sodium dichromate	< 20%	Use of Sodium dichromate for surface treatment of metals such as aluminium, steel, zinc, magnesium, titanium, alloys, composites and sealings of anodic films.	PROC 13: Surface treatment with Cr(VI)- chemical pre-treatment	basic general ventilation (1-3 ACH).	1.26 (90th percentile) Measured data correction for RPE	
0105-02	Sodium	39.7% and	Use of Sodium Dichromate as a mordant in	PROC 3: Dyeing process stage	Effective LEV in filling line and	< 0.4	

Authori-zation ID	Substance	% of Cr(VI)	Use (Spraying/platting/dyes)	WCS (chosen the ones of interest: *worst case scenario*)	RMM	Exposure estimation Air monitoring µg Cr(VI)/m^3	Exposure estimation Biomonitoring Chromium µg /L, creatinine corrected
	dichromate	24.2%	the dyeing of wool as sliver and/or yarn with dark colours in industrial settings		PPE (RPE)	Measured	1.21-3.5 (90th percentile)
0101-01	Sodium dichromate	< 0.1 %	Use of Sodium dichromate for surface treatment of metals such as aluminium, steel, zinc, magnesium, titanium, alloys, composites, sealings of anodic films	PROC 13: Surface treatment with Cr(VI) - chemical pretreatment	LEV on the side of each bath.	1.5 Modelled ART 1.5 (no RPE used)	Blood values were all below of 0.5 and urine values were all below 0.6.
0097-01	Sodium dichromate	<30%	Use of sodium dichromate for sealing after anodizing applications by aerospace companies and their suppliers	Refilling of baths liquids	ACH: natural LEV: 90% eff.	0.11 µg/m3 Modelled ART 90th perc. Freq. adjusted	
0063-03	Sodium dichromate	10- 50%	Use of Sodium dichromate for the electrolytic passivation of tin plated steel for the packaging industry.	PROC 2, PROC 13: Surface treatment with Cr(VI)- by dipping/immersion	General ventilation: Basic (1-3 ACH per hour) PPE	1.26 90th percentile following correction for RPE	
0043-02	Sodium dichromate	Cr(VI): < 20%	Use of Sodium dichromate for surface treatment of metals such as aluminium, steel, zinc, magnesium, titanium, alloys, composites and sealings of anodic films	PROC 2,13: Surface treatment with Cr(VI)- by dipping/immersio	General ventilation: Basic (1-3 ACH per hour) + LEV PPE	1.26 90th percentile Measured correction for RPE	
0099-02	Sodium dichromate	0.5- 1%	Use of sodium chromate for sealing after anodizing, chemical conversion coating, pickling and etching applications by aerospace companies and their suppliers	PROC 13: Use of sodium chromate for chemical conversion coating applications by aerospace companies and	General ventilation: Good natural ventilation + LEV - Fixed capturing hood (90% efficiency)	0.0058 Modelled ART 90th perc	
0072-05	Potassium dichromate	0.12-10%	Industrial use of a mixture containing hexavalent chromium compounds in conversion coating and passivation of circular and rectangular connectors in order to meet the requirements of international standards and special requirements of industries subject to harsh environments.	PROC 13: Plating -Manual dipping in Cr(VI) bath	General vent.: 3-10.2 ACH LEV - Fixed capturing hood	1.77×10^{-2} corrected for RPE, duration and frequency	
0072-04	Potassium dichromate	0.12-10%	Industrial use of a mixture containing hexavalent chromium compounds for the conversion of cadmium coated circular and rectangular connectors	PROC 13: Manual dipping in Cr(VI) bath	General vent.: 3-10.2 ACH LEV - Fixed capturing hood	0.0053 Modelled, ART Corrected for duration, RPE & frequency*	
0062-02	Potassium dichromate	<20%	Use of potassium dichromate for surface treatment of metals such as aluminium, steel, zinc, magnesium, titanium, alloys, composites, sealings of anodic films.	PROC 2, PROC 13: Surface treatment with Cr(VI) - by dipping/immersio n	General ventilation: Basic (1 -3 ACH per hour) + LEV	1.26 Measured, 90th percentile and correction for RPE	
0121-02	Pentazinc chromate octahydroxid	< 0.01 – 0.1%	Use of pentazinc chromate octahydroxide in stoved epoxy primer for corrosion protection of aircraft engine components in aerospace	PROC 7: Surface treatment by spraying in spray cabin / spray booth	General ventilation: Down -flow spray room RPE: Yes (with APF 400)	9.25×10^{-3} Modelled ART 5.1 RPE adjusted	

Authori-zation ID	Substance	% of Cr(VI)	Use (Spraying/platting/dyes)	WCS (chosen the ones of interest: *worst case scenario*)	RMM	Exposure estimation Air monitoring µg Cr(VI)/m^3	Exposure estimation Biomonitoring Chromium µg /L, creatinine corrected
	e		and aeroderivative applications				
0118-02	Pentazinc chromate octahydroxide	< 0.5 - 1 %	Use of pentazinc chromate octahydroxide in wash primer, fuel tank primer and aluminized primer for the purpose of corrosion protection in aeronautic applications	PROC 7: Surface treatment by spraying outside of paint -booth	General ventilation: Good natural ventilation RPE: Yes (with APF 30)	0.053 Modelled ART 1.5 RPE adjusted Freq adjusted	
0012-05	Lead sulfochromate yellow (C.I. Pigment Yellow 34)		Professional, non-consumer application of paints on metal surfaces (such as machines, vehicles, structures, signs, road furniture etc.) or as road marking	PROC 7: Pigment paint spray application in a professional spray boot	Dedicated spray room with 10 ACH (min.) RPE: APF 4001	0.09 Modelled ART 1.5 RPE adjusted	Biomonitoring for lead
0012-04	Lead chromate molybdate sulphate red (C.I. Pigment Red 104)		Industrial application of paints on metal surfaces (such as machines vehicles, structures, signs, road furniture, coil coating etc.)	PROC 7: Pigment paint spray application in a professional spray boot	Dedicated spray room with 10 ACH (min.) RPE: APF 4001	0.04 Modelled ART 1.5 RPE adjusted	Biomonitoring for lead
0012-06	Lead chromate molybdate sulphate red (C.I. Pigment Red 104)		Professional, non-consumer application of paints on metal surfaces (such as machines, vehicles, structures, signs, road furniture etc.) or as road marking	PROC 11: Pigment paint spray application in a make-shift booth on location	RPE: APF 10001	0.09 Modelled ART 1.5 RPE adjusted	Biomonitoring for lead
0057-05	Dichromium tris(chromate)	0.6% - spraying 0.37% - bath	Industrial use, of a qualified mixture of chromium trioxide by spraying or immersion, and of a qualified mixture of dichromium tris(chromate) by pen application	PROC 7: Spraying of article in contained system PROC 13: Dipping of article in ba ths treatment in the workers' far field	Technical: Good na tural ventilation; Medium level containm ent (spraying in a wa tertight containm ent with a com pletely autom a tic system); LEV - Enclosing hoods, fume cupboard	5.00×10^{-5} and 4.20×10^{-3} Modelled ART 1.5 RPE adjusted	Biomonitoring done but data not available due to confidentiality
0116-01	dichromium tris(chromate)	1-5 %	Use of dichromium tris(chromate) for chemical conversion coating applications by aerospace and defence companies and their associated supply chains	PROC 13: Use of dichromium tris(chromate) for chemical conversion coating applications by aerospace and defence companies and their associated supply chains – bath application	Ventilation: natural LEV: 90 % eff.	0.023 Modelled ART 1.5 (90th percentile)	
0032-02	Chromium trioxide	10-50%	Functional Chrome Plating	PROC 2, 13: Functional chrome plating - by dipping/imm ersion	Basic general ventilation + LEV	1.42 Measured data 90th percentile, RPE adjusted	

Authori-zation ID	Substance	% of Cr(VI)	Use (Spraying/platting/dyes)	WCS (chosen the ones of interest: *worst case scenario*)	RMM	Exposure estimation Air monitoring µg Cr(VI)/m^3	Exposure estimation Biomonitoring Chromium µg /L, creatinine corrected
0096-01	Chromium trioxide	< 1-5 %	Use of chromium trioxide for chemical conversion and slurry coating applications by aerospace companies and their suppliers	PROC 8b, 7: Use of chromium trioxide for Slurry coating - Substance preparation and surface treatment by spraying in paint booth	General ventilation: Good natural ventilation Mix coating slurry, spraying in paint booth, Tool cleaning (spray cabin) + LEV	0.27 Measured 90th perc. RPE adjusted 0.48 Modelled ART 1.5 (90th percentile) RPE adjusted	
0051-01	Chromium trioxide	CrO3: < 50%	Functional chrome plating of valves for the use in petrol and diesel engines for light-and heavy duty vehicles.	PROC 13: Functional chrome plating automatic plating line, open process	General ventilation: 5 ACH + LEV	1.02 Measured data (and supportive ART 1.5)	Biomonitoring is conducted but the employer has no access to the results of the biomonitoring.
0071-01	Chromium trioxide	<25%	Plating on Plastics for Automotive Applications (PoPAA)	PROC 13: Plating on plastics for automotive applications – automatic linE	General ventilation: Basic (1-3 ACH) + LEV	0.21 Modelled ART 1.5 (90th percentile) RPE adjusted	Biomonitoring is conducted but not submitted due to confidentiality
0034-02	Chromium trioxide	< 25 %	The use of Chromium Trioxide for a pre-treatment step (etching) in the electroplating process	PROC 13: Functional chrome electroplating - etching	Use of mist suppressant + LEV	0.019 Measured	Biomonitoring results were provided upon request by RAC but not presented in the opinion.
0054-02	Chromium trioxide	CrO3: < 5% w/w	Industrial spraying of chromium trioxide mixtures for the coating of metallic articles	PROC 7: Manual spraying	Dedicated room and dedicated opera tor LEV: Other enclosing hood (90% reduction)* General ventilation: specialised room ventila tion with more than 10 ACH PPE: air supplied respira tor with full face shield APF 100	0.38 Modelled ART 1.5 RPE and duration adjusted 0.019 Measured RPE and duration adjusted	Biomonitoring is conducted but not provided.
0057-01	Chromium trioxide		Industrial use of a mixture of chromium trioxide for the hard chromium plating of military armament	PROC 13: Dipping arm am ent parts in ba ths tre tment	Technical: Good na tural ventilation; Local exhaust ventilation fixed on the lip of the bath; Half m ask with P3 filter (EN 143 – APF 10);	2.24×10^{-3} Modelled ART 1.5 adjusted for frequency of task and RPE	Biomonitoring is conducted but not submitted due to confidentiality.
0058-01	Chromium	<1%	Industrial use of chromium trioxide for the	PROC 13 - Electrochemic al	General ventilation 5- 10 air	0.15	Upon RAC´s

Authori-zation ID	Substance	% of Cr(VI)	Use (Spraying/platting/dyes)	WCS (chosen the ones of interest: *worst case scenario*)	RMM	Exposure estimation Air monitoring µg Cr(VI)/m^3	Exposure estimation Biomonitoring Chromium µg /L, creatinine corrected
	trioxide		treatment of copper foil used in the manufacture of Printed Circuit Board	surface treatment	changes / hr LEV by lip extraction on bath	Measured Exposure estimation corrected for frequency and duration	request, the applicant provided some biomonitoring data which showed that the workers have *tolerable* chrome levels (in urine or in blood) with respect to reference value limits in occupational medicine
0072-02	Chromium trioxide		Industrial use of a mixture containing hexavalent chromium compounds in conversion coating and passivation of circular and rectangular connectors	PROC 13 - Dipping connector parts in treatment bath, in the worker's near field	General vent.: 3-10.2 ACH LEV - Fixed capturing hood	1.77 x 10^{-2} Modelled ART 1.5 Corrected for duration, RPE & frequency	
0095-04	Chromium trioxide		Surface treatment (except ETP) for applications in various industry sectors namely architectural, automotive, metal manufacturing and finishing, and general engineering	PROC 2, 13: Other surface treatment - by dipping/immersi on	General ventilation: Basic (1 -3 ACH per hour) + LEV	1.25 Measured 90th percentile Adjusted for RPE	
0095-03	Chromium trioxide	10-50%	Functional chrome plating with decorative character	PROC 2, 13: Functional chrome plating with decorative character - by dipping/immersi on	General ventilation: Basic (1 -3 ACH per hour) + LEV	3.07 Measured 90th percentile 1.54 Adjusted for RPE	
0100-02	Chromium trioxide	15%	Surface treatment for applications in the aeronautics and aerospace industries (unrelated to Functional chrome plating or Functional chrome plating with decorative character)	PROC 7: Surface treatment by spraying outside of spray booth	Basic general ventilation 3 ACH. Galvanik room exhaust ventilation: a total 22,000 m³/h are exhausted via the LEVs at the baths. This induces an air flow into the Galvanik room and towards the baths. PPE: respiratory protection - mask FFP	0.031 Measured Stationary sampling 0.55 Modelled ART 1.5 Adjusted for RPE and frequency	Blood values were all below of 0.5 and urine values were all below 0.6.
0032-04	Chromium trioxide	20-50%	Surface treatment for applications in the aeronautics and aerospace industries, unrelated to Functional chrome plating or	PROC 13: Other surface treatment – by dipping/immersion	Basic general ventilation	1.25 Measured 90th percentile,	

Authori-zation ID	Substance	% of Cr(VI)	Use (Spraying/platting/dyes)	WCS (chosen the ones of interest: *worst case scenario*)	RMM	Exposure estimation Air monitoring µg Cr(VI)/m^3	Exposure estimation Biomonitoring Chromium µg /L, creatinine corrected
			Functional chrome plating with decorative character	PROC 7: Surface treatment by spraying outside of spray booth		Adjusted for RPE 1.55 Modelled ART 1.5	
0034-01	Chromium trioxide	< 25%	The use of Chromium trioxide for electroplating of different types of substrates	PROC 13: Functional chrome electroplating with decorative character with Cr(VI)	Use of mist suppressant when technically/practically possible (no suppressant at Hemer site); LEV: Lip extraction installed on the baths, (efficiency 90%);	1.140 Measured 0.019 After ajustement for RPE	Biomonitoring (blood, serum or urine) is conducted on a regular basis but not provided.
0070-02	Chromium trioxide		Industrial use of chromium trioxide in the hard chromium coating of civilian firearms barrel bores and auxiliary parts subject to thermal, mechanical and chemical stresses	Dipping of article in the worker's far field (PROC 13)	General ventilation (6 ACH); LEV - Fume cupboard (the fixed capturing hood associated and designed with the closure of the movable shutter; efficiency: 99%)	2.60×10^{-2} Adjusted for frequency and RPE	38% of measured values were below < 0.5 µg Cr/g of creatinine, 31% were between 0.5 and 1 µg Cr/g of creatinine and another 31% were between 1 and 3 µg Cr/g of creatinine.
0066-02	Chromium trioxide	< 1 – 5 %	Surface treatment for aerospace applications for civil and military uses.	Manual surface treatment process bath application (PROC 13)	General ventilation: good natural ventilation) + LEV	<0.26 Measured (personal sampling) 0.13 (50% LOD)	Results of biomonitoring were not provided
0119-01	Chromium trioxide	<30%	Functional chrome plating of piston rods for automotive and rail applications	PROC 13 - Functional chrome plating – semi-closed plating process	5 air changes per hour LEV	0.33 Measured (static sampling)	In urine below and higher than 0.6.
0100-01	Chromium trioxide	15%	Functional chrome plating	PROC 13 - Functional chrome plating - by dipping/immersion	LEV on the side of each bath	0.66 Measured 1.9 Modelled	Blood values were all below of 0.5 and urine values were all below 0.6.
0067-01	Chromium trioxide	52 %	Use of chromium trioxide in electroplating of mechanical and electromechanical cylinders	Electroplating PROC 2,4,13	LEV	0.1 Measured 2 Modelled	<0.01 µmol/l
0055-01	Chromium trioxide	15-30%	Functional Chrome Plating	PROC 13 - Functional chrome plating - by dipping/immersion	Containment: Partial enclosure + LEV	2.1 Modelled	Urine values geometric mean =

Authori-zation ID	Substance	% of Cr(VI)	Use (Spraying/platting/dyes)	WCS (chosen the ones of interest: *worst case scenario*)	RMM	Exposure estimation Air monitoring µg Cr(VI)/m^3	Exposure estimation Biomonitoring Chromium µg /L, creatinine corrected
						ART 1.5	0.03 µmol Cr/L 90 percentile= 0.09 µmol Cr/L
0056-01	Chromium trioxide		Use of chromium trioxide as an oxidising and hardening agent in the manufacture of coloured stainless steel	Operators of colouring lines	Basic general ventilation, 1-3 ACH; LEV on some colouring and black colouring tank.	387.04 ng/m^3 (90th percentile) Range: 19 - 421 ng/m3	Urine Chemists: Mean of 1.933 Cr µmol/mol creatinine Operators of the colouring line: mean of 1.908 Cr µmol/mol creatinine seems to be more exposed than maintenance workers: mean of 1.311 Cr µmol/mol creatinine
0069-01	Chromium trioxide	20%	Functional chrome plating in closed reactor systems for the establishment of adjustable hemispherical surface structures.	PROC 13: Functional chrome plating – closed (manual) reactor system	Closed reactor system + LEV	0.71 Modelled ART 1.5 Adjusted for PPE and frequency	Results of biomonitoring were not provided
0054-01	Chromium trioxide	Chromium trioxide < 5% w/w	Industrial spraying or brush application of chromium trioxide mixtures for the coating of metallic articles subject to harsh environment	PROC 7: Manual spraying	General ventilation: specialized room ventilation with more than 10 ACH + LEV (90% reduction)	0.38 Modelled ART 1.5 Adjusted for PPE 8.20 Measured without RPE 0.082 with RPE	Workers have acceptable chrome levels in urine as: a) the Cr(VI) values for workers are comparable with reference value for unexposed people (0.8 µg/g for creatinine), b) values are below of biological Limit Value for exposed people (15 µg/g for creatinine).
0057-04	Chromium trioxide	0.37%	Industrial use, of a qualified mixture of chromium trioxide by spraying or immersion	PROC 13: Dipping of article in baths treatment in the workers' far	Good natural ventilation	6.14 × 10-3 Modelled ART 1.5 Adjusted for PPE	Results of biomonitoring were not

Authori-zation ID	Substance	% of Cr(VI)	Use (Spraying/platting/dyes)	WCS (chosen the ones of interest: *worst case scenario*)	RMM	Exposure estimation Air monitoring µg Cr(VI)/m^3	Exposure estimation Biomonitoring Chromium µg /L, creatinine corrected
				field			provided
0072-01	Chromium trioxide	0.12 - 10%	Industrial use of a mixture containing hexavalent chromium compounds for the conversion of cadmium coated circular and rectangular connectors	PROC 13: Dipping connector parts in treatment bath, in the worker's near field	General ventilation : 3ACH LEV - fixed capturing hood	0.0053 Modelled ART 1.5 Adjusted for duration, RPE & frequency	
0114–02	Chromium trioxide		The use of chromium trioxide for a pre-treatment step (etching) in the electroplating process	PROC 8: Repair and maintenance	Mechanical ventilation giving at least 1 ACH. LEV (chrome baths only).	0.37 Measured 0.05 adjusted for PPE, length of time during a shift and number of exposure days	Results of biomonitoring were not provided
0066-01	Chromium trioxide	< 20%	Functional chrome plating for aerospace applications for civil and military uses	PROC 13: Functional chrome plating- manual plating process	Good natural ventilation + LEV	< LOD (0.02) Measured 4.00×10^{-2} Modelled ART 1.5 Adjusted for frequency	Results of biomonitoring were not provided
0057-03	Chromium trioxide	10-50%	Industrial use of a mixture of chromium trioxide for the black colour hard chromium plating of exterior surface of steel weapon barrel designed for military use	PROC 13: Dipping armament parts in baths treatment	Good natural ventilation; LEV fixed on the lip of the bath; PPE: Half mask with P3 filter	0.25 µg/m^3 Measured 2.69×10^{-3} Modelled ART 1.5 Adjusted for Frequency and RPE	Results of biomonitoring were not provided
0065-01	Chromium trioxide	5 - 20%	Industrial use of a chromium trioxide based surface treatment mixture applied on safety-critical rotating components of commercial and military aircraft engines, whose failure endangers airworthiness	PROC 13: Gun spraying	General ventilation: Down flow spray room (80% reduction); Local exhaust ventilation (LEV): Primary: Horizontal/downward laminar flow booth (90% reduction) Secondary: Fixed capturing hood (90% reduction);	14.5 Modelled ART 1.5 5.8×10^{-2} Adjusted for RPE	9 out of 12 measured values were below < 1 µg Cr/g of creatinine, 2 were between 1 and 1.47 µg Cr/g of creatinine and 1 was between 1 and 3.78 µg Cr/g of creatinine.
0070-01	Chromium trioxide	20-40%	Industrial use of chromium trioxide in the hard chromium coating of military small- and medium-caliber firearms barrel bores	PROC 13: Dipping of article in the worker's near field	Technical: General ventilation (3 ACH); Low level of containment.	1.54 Modelled ART 1.5 0.46	38% of measured values were below < 0.5 µg Cr/g of creatinine,

Authori-zation ID	**Substance**	**% of Cr(VI)**	**Use (Spraying/platting/dyes)**	**WCS (chosen the ones of interest: *worst case scenario*)**	**RMM**	**Exposure estimation Air monitoring µg Cr(VI)/m^3**	**Exposure estimation Biomonitoring Chromium µg /L, creatinine corrected**
						Adjusted for RPE and frequency	31% were between 0.5 and 1 µg Cr/g of creatinine and another 31% were between 1 and 3 µg Cr/g of creatinine
0095-02	Chromium trioxide	10-50%	Functional Chrome Plating	PROC 13: Functional chrome plating – by dipping and immersion	Basic general ventilation	1.42 (90th perc.) Measured	
0068-01	Chromium trioxide	<15%	Functional chrome plating of work rolls used in the steel and aluminum industry.	PROC13 : Functional chrome plating – manual plating process	Natural Ventilation and LEV in the tanks	0.75 Measured	Range of values considering all the units <0.5 – 1.8 (µg Cr/g Creatinine)

Annex D: flame retardants risk assessment

D5.5 Substance-group specific risk assessment for flame retardants

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1 Introduction

Flame retardant (FR) is the term given to any chemical substance added to a consumer product or building material to reduce the flammability and thus improve product safety. Flame retardants can be either chemically-bound to the material of the consumer product, or chemical additives (not bound to the product material). A range of both inorganic and organic FRs are in use; however, of concern with respect to HBM4EU are the synthetic organic flame retardants. There are three primary types of synthetic organic FRs categorized based on their elemental composition, these being bromine (Br), chlorine (Cl) and phosphate (P). Since the 1970s, the primary FR compounds used were the polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCDD). However, due to concerns regarding their persistence, toxicity and bioaccumulative potential, several of them have been severely restricted and replaced by other brominated, chlorinated and organophosphate compounds (HBM4EU Scoping document, November 2017). In the group of replacement FR, aromatic as well as halogenated aromatic substructures still occur but halogenated diphenylethers seem to be absent.

An overarching goal of the European Human Biomonitoring Initiative HBM4EU is to "generate knowledge to inform the safe management of chemicals". One of the important goals based on this is to "improve chemical risk assessment through the use of HBM data" (HBM4EU WP5) and first to "map the information needs of policy makers" in this respect (HBM4EU WP4). These needs are clear from the questions in the scoping document for the Flame Retardants or FR (version November 2017) as listed in Annex 9 of the current deliverable.

There are very few straightforward questions that relate to risk assessment, the subject of the current deliverable. Only policy questions 10, 11, 12 and 14 have some components in them that are usually also part of risk assessment (exposure and toxicity). It was noted that all policy questions – including these four mentioned – are very broad. There is no specification for one or a few specific flame retardants, neither 'regulated' neither 'replacement' flame retardants.

Despite the very wide (non-specific) 'scope' of the policy questions, Chapter 5 of the scoping document highlights 20 FR for "research activities to be undertaken", based on "evidence of toxicity but a lack of HBM data". These 20 FR are listed, including their full names and CAS RN in Annex 9:

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

A bit surprising, Category A substances (PBDE's and HBCDD) are absent in this list.

In the same Chapter 5 however, in Table 4 "Listing of research activities to be carried out to answer the policy questions", several PBDE's as well as HBCDD being Category A substances were included.

Based on the policy questions, knowledge gaps with research activities needed were defined by the Chemical Group Leader and listed in Table 4 of the scoping document. They include amongst others:

- identifying time trends to check whether current regulatory structure is effectively leading to decrease in human exposure
- variance between population subgroups, both regional and **at risk**
- evaluation of methods and choice of biological matrices
- analytical methods
- HBM data quality
- screening for data gaps for regions and specific individual FRs
- screening of biobanks for Cat C substance.

In the current policy needs, risk-related issues do not seem to be of high priority. Only for PBDE's and HBCDD there was some attention given to risk-related questions (second bullet point above), but these were not part of the 20 prioritised FR in the scoping document. As to start risk assessment, not only HBM data are needed but preferably also a health-based HBM guidance value (HBM-GV) like HBM-I or BE, high priority was given to the availability of any HBM-GV before doing any further work. Another indirect way to perform more provisional risk assessment is possible when HBM data are available regarding exposure and an external guidance value (e.g. a TDI) as well as a kinetic model (simple one- or two-compartmental model or a more sophisticated PBK model). The kinetic model could then be used to back-calculate (reverse dosimetry) expected external exposure values and the predicted external exposure values could then be compared to the external guidance value. By using the risk characterisation ratio (RCR) approach and comparing the model-predicted external exposure to the external exposure guidance, a provisional risk number could be calculated. This provisional RCR could be used to focus and prioritise further work (risk management), e.g. to come up with measured instead of modelled external exposure or to focus work on specific subpopulations or specific (high provisional RCR-related) FR.

Screening the literature allowed to conclude that the information data base is varying widely from one FR to another. For the regulated FR in category A, the amount of information

regarding human exposure and hazard is relatively large. For many of the non-regulated (replacement) FR, very little to no information on exposure and/or hazard seemed available. In order to assess for which FR it would make sense to try to improve current risk assessment and/or health impact assessment, it is necessary (1a) that the substance is prioritised in the scoping document, (1b) although very much linked to 1a that there is a policy question that relates to risk assessment or health impact assessment issues for that substance, (2) as the current work intends to describe the existing risk assessment(s) that HBM data could be added to in order to see if the risk assessment(s) can be improved, that at least some kind of risk assessment (i.e. based on external exposure and external guidance value or an already existing risk assessment using HBM data) is available on the specific substance and (3) as establishing internal guidance values (HBM-GV) is out of the scope of the current Deliverable 5.5, that either an HBM GV is available or an external guidance value in combination with reverse dosimetry kinetic modelling results based on concrete HBM data or the feasibility to perform simple one-compartmental modelling (i.e. the availability of FUE as substance-specific parameter in the one-compartmental model for reverse dosimetry).

A template was developed to capture the results regarding these 3 criteria groups for each one of the 20 prioritised FR and to open the option to extend the table later with other FR on top of the 20 prioritised FR (prioritised and policy question, available external exposure-based risk assessment, and possibility to use back-calculated external exposure to feed into an RCR calculation). During the work it was felt that capturing other knowledge (some of which is available in the scoping document) about each substance in the same table would be helpful too in prioritising further work, i.e. legislative status, external exposure information, internal (HBM-based) information. Lastly, a semi-quantitative classification system was developed that was found useful for a decision-tree approach on the need and possibility for further research, one of them being HBM-based risk assessment, another one being possibly the need to develop analytical methodology or to start sampling HBM data.

The current deliverable has taken the information from the scoping document of November 2017 with respect to flame retardants (FR) as starting point for the work described in this FR part of the deliverable. The policy questions as presented in the scoping document were taken as lead for the work.

In the first phase of the project the criteria to be able to contribute to risk assessment using HBM data as well as a system to capture the results of further searches using these criteria as well as other important knowledge domains such as legislative status, analytical methods etcetera in as systematic way in tabulated form were developed. The second phase of the project encompassed initial searches for information for preliminary filling of the table as well as some concrete reference to an existing risk assessment using HBM data (HBCDD and EH-TBB) as well as attempts to improve current risk assessment (TCEP).

In summary, the group of Flame Retardants is a very diverse group of chemical structures that is grouped based on a specific technical property, i.e. presumed ‘fire retarding properties’. To explore whether HBM-based risk assessment is feasible (HBM4EU Task 5.3 goal at the basis of this report), a systematic approach seems necessary to collate information from various domains that are/can be necessary. The current report tries to establish a systematic approach of prioritisation of FR as a follow up to the scoping document with some adjustments that were deemed necessary. E.g. in contrast to the conclusions of the scoping document to leave the already regulated FR, also for regulated FR, HBM-based risk management might be needed (‘Is internal exposure decreasing

following regulatory risk reduction measures and are current exposure levels below indicative health-based HBM guidance values?'). Secondly, based on the preliminary scoring using the systematic approach and the preliminary filling of a Table to capture the results of this approach, a few FR were picked for more concrete exploration of the feasibility to incorporate HBM in and improve current risk assessments of these FR.

2 Methodology

A – Setting up the system

Based on the various domains mentioned in the scoping document, a Table was developed to enable capturing the following information regarding the various domains in a semi-quantitative way.

1. SCOPING DOCUMENT: Prioritisation in the scoping document
    a. Whether the substance is prioritised in the scoping document,
    b. Whether there is a policy question that relates to risk assessment or health impact assessment issues for that substance and
2. REGULATION: Legislative status (based on scoping document and screening several systems such as the Stockholm Convention Annexes, various REACH lists such as on restriction and authorisation and SVHC);
3. EXPOSURE:
    a. External exposure:
        i. REACH Production Volume category (tpa)
        ii. Persistency or Bioaccumulative Risk (according EFSA)
        iii. WP12 database
        iv. WP12 reverse dosimetry external exposure prediction
        v. SHINE project information on indoor exposure
    b. HBM Analytical method
    c. Internal exposure (HBM data)
4. RISK: Risk assessment(s)
    a. According to the scoping document, (external) risk assessments
    b. Additional external risk assessment in regulatory domains (such as EFSA, ECHA, US EPA, Health Canada) and found in peer-reviewed and grey literature
5. HAZARD:
    a. HBM-GV for 'internal exposure-based' risk assessment (such as, $DNEL_{internal}$, serum concentration equivalent to ADI or to TDI, internal RfD, HBM-I, HBM-II, BE)
    b. To enable provisional RCR approach (comparison to the external guidance value)
        i. a back-calculated (reverse-dosimetry) external exposure estimate
        ii. the F_{UE} to use in one-compartmental modelling to back-calculate an external exposure estimate
    c. External guidance value (Hazard (e.g. ADI, TDI, RfD, DNEL)
    d. Toxicity data
        i. Animal in vivo
        ii. Human in vitro
        iii. QSARs
    e. Epidemiological data

B – Searching and capturing information

As a first step, the information from the scoping document was used to fill the columns regarding the first information domain, i.e. 'scoping document' (Annex 10). Information retrieved was on the issues 'Category A, B, C, D or E', 'prioritised' and 'policy questions relative to Task 5.3'.

As a second step, a first screening effort for the other information domains was made using information from the scoping document, a number of REACH Annexes and activity lists as found on ECHA webpages, IPCHEM (EC JRC Information Platform for Chemical Monitoring data), a recent paper from Bajard et al. (2019). It was tried to include information as well on their relevance and solidity (refers to quality as well as number of independent data). In addition, information regarding the alerting or de-alerting trend that could be delineated from the available exposure information and/or hazard information was collected. It could be let's say 'red flag' information (biomarkers widely found in population, increasing HBM levels found over time, toxicological hazard found etc.) or 'green flag' information (biomarkers not-detected or only in small parts of the population, no concrete hazards reported). The semi-quantitative scoring system that was developed for this was based on the system used by Bajard et al. (2019). No extensive literature search on all 20 prioritised chemicals could be performed yet.

In future, aspects like relevance and reliability of the data found on the one hand and the qualitative direction of that information, e.g. "hazard alert" or "information suggests no hazard" or "determined in large part of the population" versus "detected only in very small part of the collected HBM samples" would need to be added to the system in order to improve its usefulness in priority setting for further research.

The results of the first (scoping document information) and second step were put in a table. Based on the screening effort results, one FR was prioritised for further research, i.e. **TCEP** (Category B compound as assessed previously under the European Communities Existing Substances Regulation).

For two more FR risk assessments were found – based on HBM data – while searching for information on the 20 prioritised FR. And albeit none of these two were within the 20 prioritised substances, they were included below for illustrative purposes in the remaining parts of the current work: **HBCDD** (a Category A substance) and **EH-TBB** (a Category B compound).

3 Summary of hazard characterisation

In the current work, emphasis was put on developing an integrative tool to capture available information on a wide scale of domains (from regulatory status to exposure to risk to hazard to analytics) for the vast range of FR instead of focussing on one or two specific FR upfront. Searching and summarising specifically on hazard characterisation was regarded to be outside of this scope and to focus merely for illustrative purposes on risk characterisation because of the screening level character of the exercise.

4 Summary of available exposure assessment

For similar reasons as mentioned under Chapter 3, a summary of exposure assessment of a few specific FR was regarded to be outside the current scope.

5 Risk characterisation and uncertainty analysis (for TCEP only)

The 2009 EU Risk Assessment Report (RAR, 2009) under the Existing Substances Regulation (ESR) concluded on several exposure categories: Occupational exposure, Consumer exposure and Indirect human exposure via the Environment. In that 'pre-REACH' regulatory framework on chemicals, only conclusion 'iii' meant 'at risk'.

For workers, risks were found for inhalation as well as dermal exposure at the workplace.

European Union Risk Assessment Report

TRIS (2-CHLOROETHYL) PHOSPHATE, TCEP

CAS-No.: 115-96-8
EINECS-No.: 204-118-5

RISK ASSESSMENT

July 2009

FINAL APPROVED VERSION

Table 4.1.3.2.H: Ranking of the critical exposure levels for TCEP with respect to inhalative exposure at the workplace

Exposure scenario		Exposure level in mg/m³	Carcino-genicity	Repeated dose toxicity, systemic	Fertility	Acute toxicity
			Critical exposure level in mg/m³			
			0.2 mg/m³	1 mg/m³	16 mg/m³	70 mg/m³
3a	Use of formulations with spray application	8.3	iii	iii		
1	Production	1.2	iii	iii		
2	Processing to formulations	1.2	iii	iii		
3b	Use of formulations without aerosol formation	1.2	iii	iii		

(1) blank fields: conclusion ii

Table 4.1.3.2.I: Ranking of the critical exposure levels for TCEP with respect to dermal exposure at the workplace

Exposure scenario		Exposure level in mg/p/d	Carcino-genicity	Repeated dose toxicity, systemic	Fertility	Acute toxicity
			Critical exposure level in mg/p/d			
			2 mg/p/d	10 mg/p/d	160 mg/p/d	15000 mg/p/d
3a	Use of formulations with spray application	2500	iii	iii	iii	
1	Production	420	iii	iii	iii	
2	Processing to formulations	420	iii	iii	iii	
3b	Use of formulations without aerosol formation	210	iii	iii	iii	

(1) blank fields: conclusion ii

Assuming a body weight of workers of 70 kg, the **critical exposure levels** as provided in the table above in mg/p/d would correspond to **0.029, 0.14, 2.3 and 214 mg/kg bw/d.**

Regarding consumer exposure, it was concluded (conclusion 'iii' in the following table) that only babies were 'at risk' for exposure to TCEP (Margin of Safety of only 50):

Table 4.1.3.3
Repeated dose toxicity of TCEP:
Margins of Safety for consumer exposure via different routes

Route	Exposure (µg/kg bw/d)	MOS *)	Conclusion
Inhalation			
Adults	0.4	30000	(ii)
Children	0.96	12500	(ii)
Dermal			
Adults	~ 4	3000	(ii)
Children	10	1200	(ii)
Oral			
Adults	0.0033	$>3 \cdot 10^6$	(ii)
Children	0.2	60000	(ii)
Babies	240	50	(iii)

*) The MOS was derived by using the oral LOAEL of 12 mg/kg bw/d (kidney effects)

The oral **LOAEL** for **kidney effects** of **12 mg/kg bw/d** from this table will be used further on to compare to reconstructed (modelled) external exposure values based on HBM measurements.

Regarding indirect exposure via the environment, it was concluded there was no (current) need for risk reduction beyond measures already applied.

6 Improving the RA by biomonitoring

In this chapter, information is provided on only one substance for which risk assessment could be performed using HBM data (TCEP) and on two substances for which a risk assessment was found based on HBM data (HBCDD and EH-TPP).

6.1 Evaluation of the available biomonitoring data

TCEP [115-96-8]

TCEP was prioritised in the scoping document as Cat. B substance (see Chapter 1 'Introduction). A limited amount of HBM data related to TCEP was found:

Schindler et al (2009), also referenced in HBM4EU AD 12.5 published a range of TCEP (tris-(2-chloroethyl)-phosphate) human biomarker levels of BCEP (bis-(2-chloroethyl)-phosphate; the de-phosphorylated TCEP) HBM levels as found in urine of 16 females and 14 males in southern Germany aged 11-68 years (median 39.5 years), being <LOD (0.1 µg/l) – 27.5 µg/l and a median value smaller than LOD (0.1 µg/L).

In addition, data from children in 63 day-care centres in Germany were found (Fromme et al., 2014). The levels found were significantly lower than the worst-case level in adults in southern Germany as listed above. In the children's urine samples, the metabolite DBEP had median values (95th percentiles) of 2.0 µg/l (10.7 µg/l), respectively.

Based on the values of Schindler, an external exposure value range was calculated and presented in HBM4EU AD12.5: mean intake of adults in the range of 0.02 µg/kg/d and the worst-case estimate of almost 1 µg/kg/d. Based on the values of Fromme et al., the intake range in children would be a bit lower but in the same order of magnitude as the intake range in adults (AD12.5; Fig. 16).

In addition, it was tried to predict external exposure using simple one-compartmental kinetic modelling as presented by Angerer et al., 2011. The only substance-specific parameter needed is the proportional urinary excretion fraction, F_{UE}, to be used in the following formula that can be used to calculate "TI" the other way around

$$C = \frac{\mathrm{TI} * \mathrm{BW} * F_{\mathrm{UE}}}{V_{24\,\mathrm{h}}}$$

where *C* is the concentration of the substance (or a metabolite biomarker) in 24 hrs urine, TI is the tolerable intake in mg/kg bw/d, BW is the body weight in kg, F_{UE} is the proportional urinary excretion fraction (of the metabolite BCEP in this case) and V_{24h} is the volume of urine produced in 24 hrs.

This can be re-ordered to give

$$Intake = \frac{C * V24h}{BW * FUE}$$

Where *Intake* is the intake in mg/kg bw/d, BW is the body weight in kg, F_{UE} is the proportional urinary excretion fraction, V_{24h} is the volume of urine produced in 24 hrs and *C* is the concentration of the substance (or a metabolite biomarker) in 24 hrs urine. Modelled values

for intake are inversely related to FUE. The smaller the FUE, the larger the model-predicted intake.

Unfortunately, the F_{UE} could not be traced with confidence in literature nor easily calculated based on information found in literature. Albeit Zhang et al (2018) claimed that they calculated F_{UE} for TCEP based on 'Total Clearance Rate (TCR)' in human liver microsomes and human S9 incubations as published by Van den Eede et al. (2013). The values they report in the supplementary data to their paper are 0.07 and 0.13, respectively. Unfortunately, it is not clear how they came from TCR values to F_{UE}., it seems they put the F_{UE} equal to TCR, which is clearly not justifiable as one is an *in vivo* term, the other one an *in vitro* determined property which is very much dependent on issues like time, linearity, saturation, co-factor.

More strongly, Van den Eede et al. (2013) themselves state "Limitations of this study include its exclusively qualitative character, as neither the metabolite concentrations could be accurately determined for the phosphate diesters, the not steady-state incubation conditions and the lack of a larger range of substrate concentrations." Furthermore, they also note limitations of their study due to "differences between *in vivo* and their *in vitro* experimental conditions". They also state that "Further quantitative determination of the metabolite formation, as well as *in vivo* confirmation of the metabolites, is necessary". Zhang et al (2018) used HBM measurement levels to calculate health risks using the F_{UE} , the value of which is disputable as described above.

In a US Consumer Products Safety Commission statement with an underlying report by TERA (US CPSC, 2015), another metabolite than BCEP was mentioned to be found (DCEP) in human biomonitoring urine samples, i.e. DCEP. DCEP was found in a US data set from non-smokers in Califoria at mean levels of 0.76 ng/L. BCEP was found in spot urine samples from children in Germany at much higher concentrations, i.e. mean levels of 400 ng/L. Unless the ration between various metabolites in children is very much different from that in adults, this strongly indicates that *in vivo* the FUE for BCEP can not be as high as 0.07. Assuming the BCEP and DCEP represent total intake of TCEP at steady state levels (BCEP + DCEP = 100% TCEP intake) the FUE for BCEP would be 0.63 / (200 + 0.63) = 0.003. This would mean a **23-fold and 43-fold lower FUE** than the FUE values of 0.07 and 0.13, respectively, as based on the TCR in Van den Eede et al (2013).

Table 2-9. TCEP Biomonitoring Data

Country (location)	Tissue/fluid	Concentrations[1]	Reference	Notes
United States	Urinary metabolite BCEP	Mean: 0.76 ng/l Median: 0.63 ng/l Max: 2.1 ng/l	Dodson et al., 2014	Samples collected from 16 non-smoking adults living in northern California.

Country (location)	Tissue/fluid	Concentrations[1]	Reference	Notes
Germany	Urine metabolite DCEP	Mean: 400 ng/l (0.4 µg/l) Median: 200 ng/l (0.2 µg/l) Range: 100-13,100 ng/l <0.1-13.1 µg/l 95th: 1,600 ng/l (1.6 µg/l)	Fromme et al., 2014	Spot urine samples from 312 children attending daycare centers that were also measured for air and dust concentrations

However, it was found not regarded justifiable to use these data regarding two different metabolites in two completely different studies to elaborate an FUE value.

When nevertheless the one-compartment modelling is used with the uncertain F_{UE} and the same southern German urinary BCEP data (Schindler et al., 2009) to back-extrapolate exposure, higher external exposure levels (9.82 µg/kg/d) than when using the PBK-modelling approach from AD12.5 as described in the beginning of this chapter (1 µg/kg/d). Using urinary data from children in day-care centres (Fromme et al., 2014) with the one-compartmental modelling approach would even provide higher external exposure levels expressed per kg bw as children are considerably smaller (in that study 10 kg – 34 kg).

No HBM-GV was found for TCEP.

A provisional chronic oral reference dose (chronic p-RfD) was derived by US EPA (2009), i.e. 0.007 mg/kg bw/d and is used below in Chapter 6.2 in a risk assessment context.

HBCDD isomers [3194-55-6, 25637-99-4, 1093632-34-8]

A HBM-GV was found for HBCDD. But HBCDD was not within the 20 highlighted FRs in the scoping document. Therefore, no HBM measurement data were searched for this compound.

EH-TBB [183658-27-7]

A HBM-GV was found for EH-TBB (2-ethylhexyl-2,3,4,5 tetrabromobenzoate), i.e. in Hays and Kirman (2017). Hays and Kirman referred as well to at least three data sets of HBM data (see Chapter 6.2).

6.2 Risk assessment for the sample populations

Useful information to illustrate use of European HBM-data in a human risk assessment context was found for three substances:

TCEP

Using a worst case estimated exposure value of 1 µg/kg/d as taken from AD12.5 (see Chapter 6.1), and a US EPA chronic p-RfD (provisional chronic oral reference dose) of 0.007 mg/kg/d (7 µg/kg/d), a preliminary risk characterisation ratio (RCR) of 0.001/0.007 = 0.14 is resulting. Similarly, the same worst case estimated exposure value of 1 µg/kg/d can be used to compare to the LOAEL as used in the risk characterisation in the ESR TCP report as referenced in Chapter 5, i.e. 12 mg/kg bw/d. This provides a provisional MOS of 12/0.001 = 12000. This indicates that the internal exposure levels as published by Schindler et al. (2009) for a population in Germany do not indicate further research (RCR<1; MOS>1000). This is in line with the conclusion for adults and children in the EU RAR (EU RAR, 2009) the RCR was calculated for adults.

Higher external exposure levers (9.82 µg/kg/d) are obtained when using the one-compartmental modelling approach in which the (uncertain) F_{UE} is used based on the same southern German urinary BCEP data. An RCR of 9.82 / 7 = 1.4 is obtained and a MOS of 12000 / 9.82 = 1222 (using the same p-RfD and EU ESR LOAEL as used in the paragraph above, respectively). Especially the RCR of 1.4 could warrant some further scrutiny although based on the worst-case urinary BCEP level were the median was at least a factor of 275 lower.

In China however, TCEP exposure seems to be considerably higher. Zhang et al (2018) used similar methodology using BCEP as major metabolite of TCEP of urine samples from population in several Chinese cities. In summary: “The total daily intake (TDI) of tris(2-chloroethyl) phosphate (TCEP) and….. was estimated from daily urine excretion rate and the fraction of OP metabolized in human liver microsomes (TDIHLM) or S9 fraction (TDIS9). The intake estimates showed that Chinese residents were exposed to TCEP from 96.9 to 46,700 (or 52.2 to 25,200) ng/kg bw/day. Depending on the reference dose, we found that approximately 5% of the individuals exceeded the limit (i.e., 2200 ng/kg bw/day) for TCEP intake.”. However, as discussed above in Chapter 6.1, the validity of the back-extrapolated external exposure predictions could be disputed.

The reference dose of 2200 ng/kg/d or 2.2 µg/kg/d as had been taken from Van den Eede et al (2011) is about a factor of 3 below the US EPA PPTRV of 0.007 mg/kg/d (7 µg/kg/d) as indicated above. As this is a rather small difference between back-extrapolated external exposure and provisionally presumed safe external exposure, this would indicate a priority for follow-up work.

HBCDD

For **HBCDD** (hexachlorocyclododecane or HBCD) one paper was found for biomonitoring-based risk assessment (Aylward and Hays, 2011). This is mentioned purely for illustrative purpose. No HBM measurement data were searched as not prioritised in the scoping document.

https://www.sciencedirect.com/science/article/pii/S1438463911000277?via%3Dihub

“Recent risk assessment evaluations from Health Canada and the European Union have **identified points of departure of 10 and 20mg/kg day**, respectively, from rat repeated

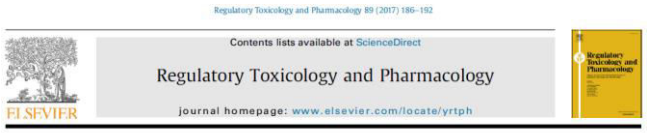
dose studies. The **corresponding** measured or estimated **lipid-adjusted tissue concentrations** in the laboratory animals at these points of departure range from **120,000 to 190,000 ng/g lipid**. In comparison to these concentrations, the **biomonitored human serum and milk concentrations** indicate **margins of exposure (MOEs) of 6000 to more than 100,000**, which are greatly in excess of target MOE values."

"Because the exposure pathways for HBCD may be varied and exposure estimation uncertain, biomonitoring for HBCD in humans shows promise as a means of reflecting integrated human exposures to HBCD with lower uncertainty than through estimation of external exposures via multiple pathways."

This illustrates that HBM can help improve risk assessment. In the case of HBCDD, data suggested that populations from which HBM data were used (to be checked in full paper which countries) are not at risk.

EH-TBB

Although there were no policy questions for **EH-TBB** (or TBB; CAS RN 183658-27-7; 2-ethylhexyl-2,3,4,5-tetrabromobenzoate) as Category C substance, it has been included here just to illustrate the process:



Risk assessment and Biomonitoring Equivalent for 2-ethylhexyl-2,3,4,5 tetrabromobenzoate (TBB) and tetrabromobenzoic acid (TBBA)

Sean M. Hays*, Christopher R. Kirman

Table 5
Derivation of Biomonitoring Equivalent.

Derivation step	Age group (years)			
	3–<6	6–<10	10–<18	> = 18
POD, mg/kg-d			91	
Human-equivalent dose, mg/kg-d[a]			25	
UF_A[b]			3	
Human-equivalent POD, mg/kg-d			8.3	
Fue, unitless			0.6	
V_{24}, ml/kg-d	30	25	20	20
CR_{24}, g cr,kg-d	16	19	21	20
BE_{POD}, mg/L	170.0	200.0	250.0	250.0
BE_{POD}, mg/g cr	310.0	260.0	240.0	250.0
UF_H			10	
Uf_d			10	
RfD (mg/kg-d)			0.083	
BE, mg/L	1.7	2.0	2.5	2.5
BE, mg/g cr	3.1	2.6	2.4	2.5

[a] HED calculated as POD divided by $(BW_{rats}/BW_{humans})^{(1-3/4)}$ BW of rats of 0.42 kg and humans of 70 kg.
[b] Uncertainty Factors; UF_A interspecies, UF_H intraspecies, UF_d database deficiency.

Unfortunately, no HBM data for EH-TBB or metabolite(s) were found in IPCHEM. Hays and Kirman reported on the Hoffman et al (2014) study presenting data with respect to the general population: "Using the BE of 2.5 mg/L and the mean ($5.3x10^{-6}$ mg/L) and maximum ($340x10^{-6}$ mg/L) concentrations of TBBA in urine reported by Hoffman et al. (2014), the MOS is determined to be approximately 450,000 at the mean exposure levels and approximately 7000 at the maximum exposure (HBM) levels, respectively".

Butt et al. (2014) measured TBBA in mothers and paired children urine, children having higher levels of TBBA in urine than their mothers. For mothers, no geometric mean was available (detection frequency was <50%), the maximum level measured was $62.2x10^{-6}$

mg/L. The children, ages 1-5 years, had geometric mean and maximum TBBA concentrations of $7.4x10^{-6}$ and $84.9x10^{-6}$ mg/L. Hays and Kirman (2017) calculated for the exposure of these children a MOS of 230,000 and 20,000 at the mean and maximum reported concentrations, respectively.

According to Hays and Kirman (2017), "The MOS of ~450,000 calculated here for TBB via the mean measures of TBBA in urine for adults and approximately 230,000 for children puts TBB amongst the chemicals with the largest MOS of those interpreted using the BEs (Aylward et al., 2013). In fact, the largest MOS measured to date using the BEs has been ~1000. This would suggest the MOS of TBB in the environment at the current time is extraordinarily large. This suggests current uses of TBB have little potential for adverse impacts to public health and thus low priority for risk management."

7 Discussion and conclusions

The overview table in Annex 10 provides an overall picture of information regarding "priority given and category attributed in the scoping document", "the regulatory status" (REACH SVHC etc), "exposure" (external and internal), "risk" (external and internal), "hazard characterization" (including external and internal health-based guidance values (TDI, RfD, DNEL, HBM-I, HBM-II, BE). Some domains (e.g. the exposure domain of information) can be very broad and extended and/or specified in future such as with environmental monitoring data, drinking water concentration data, consumer product content data, dust content, external exposure data, internal exposure data (HBM), animal and human kinetic data.

Furthermore, we are including in the considerations as well option for complementary work with other WP's and Tasks, in concrete WP 9 (Biomarker selection as prophase to actual new HBM data collection), Task 10.4 (statistical analysis and derivation of reference values), Task 12.1 (integrated exposure modelling, i.e. multimedia modelling combined with PBK modelling) and Task 12.2 (PBK modelling and reverse dosimetry for exposure reconstruction).

It was not feasible to address relevant policy questions for FR due to a variety of reasons: The policy questions were not clearly risk-related but more related to temporal trends, differences in exposure between subpopulations etc, they were rather unspecific and did not mention any specific FR (where the group of FR is very wide in chemical structures as well a numbers within each basic structure) and for most if not all of the 20 substances that were prioritised in the scooping document, insufficient information appeared to be available for a complete risk assessment (either HBM exposure data and/or guidance values external or internal and/or kinetic modelling for back-extrapolation of internal exposure measurements).

In some more detail: an actual improvement of current (external and/or internal exposure-based) risk assessment for relevant FR (prioritised in the scoping document) was not feasible due to the following reasons:

- No HBM-GV available for most of the 20 prioritised FR.
- A HBM-GV was available (HBCDD), i.e. a Biomonitoring Equivalent, but it was already part of a risk assessment that included the HBM-GV and on top of this, Margins of Exposure (MOEs) calculated were huge so no reason for further fine-tuning. Further work might become indicated if in future the HBM-GV might be lowered by including human epidemiological data (as the current HBM-GV in the Hays and Kirman (2017) paper is based on animal studies).
- For TCEP a preliminary risk assessment was performed using the risk characterisation ratio approach based on model predicted (reverse-dosimetry) exposure levels from a German study (from AD 12.5). The outcome (RCR < 1) was in line with previous conclusions using external exposure-based risk assessment in the EU RAR on TCEP (2008). The reverse-dosimetry modelling results were used as such and could not be further scrutinised (the materials and methods description provided in AD 12.5 was rather limited). Further work on other organophosphorus flame retardants will probably be undertaken in WP12 soon and TCEP might be investigated if more HBM data become available. Simple compartmental modelling to back-calculate the exposure based on urinary excretion of biomarker TBEP was not possible due to the lack in the literature of the F_{UE} (proportional urinary excretion fraction).
- A BE value was available for EH-TBB but it was already part of a risk assessment based on internal exposure so improvement as such could not be investigated. Furthermore, the Margin of Safety (MOS) values were quite large and the biomarker used is probably different from the biomarker that might have been used in European HBM campaigns (to be checked). Children appear to be exposed at higher internal levels (at least an important metabolite, being TBBA, was found more often and in higher concentrations in the urine of children than in their mother's urine.

7.1 Recommendations for the regulatory risk assessment

As FR are a group of chemicals based on use properties and not on chemical structure and policy questions were not specifically addressed to one or a few FR, a lot of time was spent to structure.

Based on the tabled overview some suggestions can be given for a complex group of chemical substances like FR. There does not seem to be a very clear common core chemical structure that determines whether a substance would have flame retardant or fire-retardant properties except very likely at least relative absence of reactivity for oxidation. The definition of a fire highlight that we are talking about contains oxidation ("fire is oxidation with the appearance of flames"). Flame retardants often, if not always, contain carbon-halogen bonds (C-F, C-Cl and C-Br), organophosphorus and/or organonitrogen moieties. We know that some PFAS with many C-F (carbon-fluorine) bonds have been used as flame retardants in the past (PFOS and PFOA). Bill AB-2998 on flame retardants (banning the use of FR in several consumer products like furniture) that recently passed US California legislature, covers *halogenated*, *organophosphorus* and *organonitrogen* flame retardants.

Therefore, and to start bringing some structure in the strategic approach on where to look for what in this 'use-defined' class of chemical substances, a structured overview table was

prepared with input from the Chemical Group Lead on Flame Retardants, i.e. Masaryk University.

In order to provide a clearer overall picture of the available information in the various domains (regulations, exposure, risk, hazard, analytics) on the wide group of FR, which could help prioritising (risk management) in order to focus concrete risk assessment work based on factual information, it is recommended to continue working with the template table as presented in Annex 10, to evaluate and where indicated, improve it, and to continue to search information on a wider group of FR and to capture this information in the improved tabulated overview.

7.2 Future prospects

If found helpful by other partners involved in FR activities within HBM4EU, it is envisaged that for any further alignment of activity planning with HBM4EU on Flame Retardants the overview table is used and regularly updated.

In order to facilitate back-extrapolation of measured biomarker levels in urine to predicted external exposure, the one-compartmental modelling algorithm could be very helpful. Unfortunately, determination of the substance-specific proportional urinary excretion fraction F_{UE} is not trivial using the generally available data and approaches as one needs quite solid data on the external exposure. One way forward would be human volunteer studies using microdosing with extremely low amounts of the chemical substance, below the levels that humans, including children, are exposed to every day. The Harada et al (2016) paper in PLOS is a recent example that describes the use of human microdosing in the field of establishing human toxicokinetics in the area of pesticides (neonicotinoids).

8 References

Note: References used to prepare the table in the Annex (Chapter 9) not included here.

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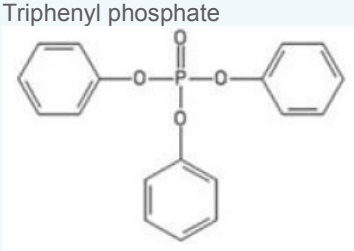
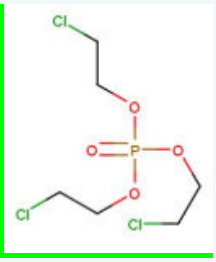
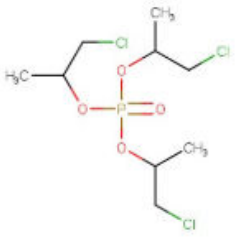
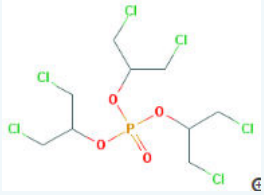
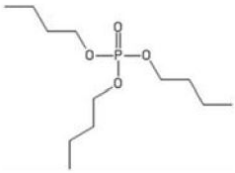
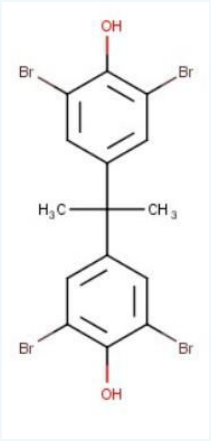
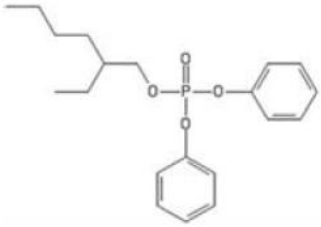
9 Annex: Policy questions and Scoping Document FRs

Table 1: from Scoping Document November 2017: Substances included in the substance group, listed according to availability of toxicology and human biomarker data, in category (Cat.) A, B, C, D, E substances. For some compounds, 2D-structures were added

Cat.	Abbreviation/ Acronym	Systematic name	CAS No.	Regulation
A	HBCDD	Hexabromocyclododecane	3194-55-6, 25637-99-4, 1093632-34-8	On REACH Authorisation List and listed on the Stockholm Convention
B	TPHP	Triphenyl phosphate 	115-86-6	Registered under REACH under the 1000-10000 T/y tonnage band and under CoRAP (suspected ED, consumer use High (aggregated) tonnage, Wide dispersive use)
	TMPP	Tricresyl phosphate	1330-78-5	Registered under REACH, entered onto CoRAP for evaluation based on High (aggregated) tonnage, Suspected PBT/vPvB, Wide dispersive use.
	TCEP	Tris-2-chloroethyl phosphate 	115-96-8	SVHC (Toxic for reproduction (Article 57c)) all uses require an Authorisation under Annex XIV of REACH from 21/08/2015. Being considered for a restriction under Article 69(2)
	TCIPP/TCPP	Tris(1-chloro-2-propyl) phosphate	13674-84-5	Registered under REACH

Cat.	Abbreviation/ Acronym	Systematic name	CAS No.	Regulation
				
	TDCIPPTDCP	Tris(1,3-dichloropropyl)phosphate 	13674-87-8	Registered under REACH, Entered onto CoRAP for evaluation in 2019 as potential endocrine disruptor
	TNBP	Tri-n-butyl phosphate 	126-73-8	Registered under REACH, Entered onto CORAP for evaluation in 2012 based on CMR, High (aggregated) tonnage, Wide dispersive use
	TBBPA	Tetrabromobisphenol A 	79-94-7	Registered under REACH under the 1000-10000 T/y tonnage band and under CoRAP (suspected PBT/vPvB, endocrine disruptor, consumer use, exposure of environment, etc.)
	TBOEP	Tri(2-butoxyethyl) phosphate	78-51-3	Registered under REACH under 1000-10000 T/y tonnage band
	EH-TBB	2-ethylhexyl-2,3,4,5-tetrabromobenzoate	183658-27-7	None

Cat.	Abbreviation/ Acronym	Systematic name	CAS No.	Regulation
C	TEHP	Tris(2-ethylhexyl) phosphate	78-42-2	Registered under REACH under 1000-10000 T/y tonnage band
	EHDPP	2-ethylhexyl diphenyl phosphate 	1241-94-7	Registered under REACH under 1000-10000 T/y tonnage band
	DDC-DBF	Dechlorane 602 (1,2,3,4,6,7,8,9,10,10,11,11-Dodecachloro-1,4,4a,5a,6,9,9a,9b-octahydro-1,4:6,9 dimethanodibenzofuran)	31107-44-5	Not registered under REACH
	2,4,6-TBP	2,4,6-tribromophenol	118-79-6	Not registered under REACH but under CoRAP (suspected PBT/vPvB, CRM, High (aggregated) tonnage, High RCR, Wide dispersive use)
	V6	2,2-bis(chloromethyl)trimethylenebis[bis(2-chloroethyl) phosphate]	38051-10-4	Registered under REACH under the 100-1000 T/y tonnage band
D	ip-TPP	Isopropyl triphenyl phosphate	68937-41-7	Registered under REACH under the 1000-10000 T/y tonnage band
	TDBPP	Tris(2,3-dibromopropyl) phosphate	126-72-7	Restricted under REACH

Short description of risk assessment needs, policy questions to be covered, and goals:

The scoping document on FR (version November 2017) lists the following policy-related questions:

1. What are current HBM levels of legacy/regulated FRs (e.g., PBDEs and HBCDD)? How do these compare to any historical records? Is the current legislative framework and proposed actions leading to a significant decline in restricted compounds and is this uniform across the EU?
2. What is the exposure of the European population to current use FRs? In particular, what is the exposure of sensitive sub-groups (e.g., infants and children)?
3. How do the levels of legacy FRs compare to levels of new/emerging FRs? Is any temporal or spatial trend observed? Can we relate this to use patterns and/or production volume?
4. How does exposure to FRs differ between adults and children, males and females?
5. How does exposure differ by geographic area within Europe? Do countries/regions have different FR exposure levels?
6. Are there one or more occupationally exposed sub-groups? What occupations are associated with high exposure to FRs?
7. Is elevated exposure to FRs associated with particular consumption patterns or lifestyles?
8. What are the relevant exposure pathways for FRs, e.g., diet, air, water, indoor environmental exposure?
9. Do certain flame retardants co-occur in HBM matrices?
10. What current information is available regarding toxicity of FRs, both as individual compounds and as the mixtures of FRs typically occurring in indoor environments and diet?
11. Can exposure to FRs be linked with any adverse health effects?
12. What are the population groups most at risk?
13. As FR market shifts towards replacement/alternative FRs, does human exposure reflect that trend? E.g., DBDPE as replacement for BDE-209;
14. What additional FRs should be prioritized for further information regarding exposure and/or toxicity? How can use and risk information be combined to identify and prioritize knowledge gaps for further assessment?
15. Can reference values be established for any FRs?

10 Annex: Overview information domains (regulations, exposure, risk, hazard) on FR

DATE : 2019-02-15

Substance information			Scoping docs			Regulation						Exposure												Risk								Hazard			
Abbreviation	CAS RN	Type	Category	Prioritized (Chapter 5)	Policy questions for T5.3?	Stockholm Convention	REACH Restriction/Authorisation	REACH SVHC	REACH PBT	REACH RMOA	REACH CoRAP	REACH Production Volume	CoRAP	PB risk according to EFSA	Temporal/ geographic info available?	WP12 environmental data in database?	IPCHEM environmental data available?	WP12 dietary data in database?	EFSA food evaluation?	WP12 consumer exposure in database?	SHINE data available?	Sufficient data for regulatory decision?	**HBM analytical method available?**	**HBM exposure data available?**	External HBGV available?	Risk identified?	MOE ref value available?	Risk identified?	MOS ref value available?	Risk identified?	**HBM-HBGV available?**	**Risk identified?**	Data availability & concern	In vivo data	In vivo + ToxCast combined	ToxCast data
TPHP, TPP	115-86-6	P	B																																
TMPP, TCP	1330-78-5	P	B																																
TCEP	115-96-8	P	B																																
TCIPP, TCPP	13674-84-5	P	B																																
TDCIPP, TDCPP	13674-87-8	P	B																																
TNBP	126-73-8	P	B																																
TBBPA	79-94-7	Br	B																																
TBOEP, TBEP	78-51-3	P	B																																
TEHP	78-42-2	P	C																																
EHDPP, EHDPHP	1241-94-7	P	C																																
DDC-DBF	31107-44-5	Cl	C																																
2,4,6-TBP	118-79-6	Br	C																																
V6	38051-10-4	P	C																																
ip-TPP	68937-41-7	P	D																																
TDBPP	126-72-7	P	D																																
DBNPG	3296-90-0	Br	E																																
TDBP-TAZTO	52434-90-9	Br	E																																
RBDPP, RDP	57583-54-7	P	E																																
EBTEBPI	32588-76-4	Br	E																																
Melamine polyphosphate	20208-95-1	P	E																																
Melamine polyphosphate	218768-84-4	P	E																																

Highest priority for evaluation in T5.3 ↑			yes			high concern						sufficient										yes (standardized	yes + expected*	yes	yes	yes	yes	yes	yes	yes	yes	sufficient; toxicity			
Lowest priority for evaluation in T5.3 ↓			no			concern						available										yes (varying)	yes **	no	no	no	no	no	no	no	no	little; toxicity			
						little concern						little										limited	expected*	not evaluated								little; 'de-alerting'			
						no use registered						scarce										no	no									sufficient; 'de-alerting'			
						not listed / no assessment						no																				insufficient			
						not evaluated						not evaluated											*expected = new HBM4EU data upcoming									not evaluated			
																							**yes = IPCHEM + HBM4EU Repository												

This table was prepared in collaboration with Masaryk University. Information on hazard from a manuscript by Lisa Melymuk submitted were kindly provided.
See Annex 11 for explanation of various terms in the domains.

11 Terms used in overview table

Parameter	Explanation
Identity	
Abbreviation	Acronym used for full name
CAS RN	Chemical Abstract Service Registry Number
Type	Phosphorylated, chlorinated or brominated FR
Scoping documents	
Category	Categories A-E; see scoping document for definition
Prioritized (Chapter 5)	This sub-table now contains only substances prioritised by the CGL in the scoping document
Policy questions for T5.3?	Are there any policy questions related to this specific substance addressed to T5.3?
Regulations	
Stockholm Convention	Listed on one of the Annexes to the Stockholm Convention
REACH Restriction/Authorisation	On REACH Annex XIV (Authorisation) or Annex XVII (Restriction); will be split in next version
REACH SVHC	Identified as SVHC under REACH
REACH PBT	Identified as (suspected) PBT or vPvB under REACH
REACH RMOA	Under current Risk Management Option Anaylysis
REACH CoRAP	Listed in the Community Rolling Action Plan due to suspected endocrine disruption and/or CMR properties
Exposure-related information	
REACH Production Volume	As indicated in the REACH Registration Dossier. From 100-1000 tpa (“concern”) to 1000-10000 tpa (“high concern”)
CoRAP	Listed on the CoRAP as of concern because of wide dispersive use
PB risk according to EFSA	Risk for persistency and/or bioaccumulation as evaluated by EFSA in a Scientific Opinion
Temporal/ geographic info available?	Information based on scoping document
WP12 environmental data in database?	HBM4EU WP12 DATABASE (https://hbm4eu.enve-lab.eu)
IPCHEM environmental data available?	Environmental Monitoring data present in IPCHEM
WP12 dietary data in database?	HBM4EU WP12 DATABASE (https://hbm4eu.enve-lab.eu)
EFSA food evaluation?	Scientific opinion of EFSA has been published and is available

	Parameter	Explanation
		online.
	WP12 consumer exposure in database?	HBM4EU WP12 DATABASE (https://hbm4eu.enve-lab.eu)
	SHINE data available?	Does it mean that data on presence in indoor environment (dust) are already published on indicated chemicals?
	Sufficient data for regulatory decision?	HBM4EU WP12 DATABASE (https://hbm4eu.enve-lab.eu)
	HBM analytical method available?	Based on information in scoping document
	HBM exposure data available?	HBM Exposure data available in IPCHEM and/or HBM4EU Repository. In future, an additional column might be added referring to papers or grey literature (reports)
Risk-related information		
	External HBGV available?	External Health-Based Guidance Value (TDI, RfD etc) available
	Risk identified?	Exposure > HBGV
	MOE ref value available?	Minimal MOE value available based on assessment factors needed
	Risk identified?	MOE < Reference MOE
	MOS ref value available?	Minimal MOS value available based on assessment factors needed
	Risk identified?	MOS < Reference MOS
	HBM-GV available?	Is there a HBM-GV? Like HBM-I or BE?
	Risk identified?	HBM Level > HBM-GV
Hazard-related information		
	Data availability & concern	How much information on hazard is available AND does it indicate a concern?
	In vivo data	Do in vivo toxicity data raise concern for the substance?
	In vivo + ToxCast combined	Integrated hazard indication based on both in vivo and ToxCast data
	ToxCast data	Do ToxCast data raise concern for the substance?

Annex E: PAHs risk assessment

D5.5 Substance-group specific risk assessment for Polycyclic Aromatic Hydrocarbons (PAHs)

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1 Introduction

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

Humans can be exposed to PAHs through different routes (Tschersich et al., 2018). 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). Regarding occupational exposure to PAHs, it can 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).

PAHs are well-known to be hazardous for human health and the environment. Many of them are classified as carcinogenic, mutagenic, and reprotoxic in the categories 1A, 1B, or 2 according to the CLP Regulation (EC 1272/2008). As very common environmental contaminants, PAHs can be found in a number of consumer products and are restricted due to their high concerns. Eight PAHs are included in http://www.chemsafetypro.com/Topics/EU/REACH_annex_xvii_REACH_restricted_substance_list.html for restrictions purposes in tyres/extender oils, articles supplied to the general public and children's articles (https://echa.europa.eu/fr/substance-information/-/substanceinfo/100.239.209). Some PAHs congeners are included also in the Candidate List of substances of very high concern (SVHC) for Authorisation in accordance with Article 59(10) of the REACH Regulation (https://echa.europa.eu/candidate-list-table). Different PAHs congeners are listed below, where restricted PAHs are designated with the superscription [1] and the SVHC candidate PAHs are designated with the superscription [2].

1. Anthracene[2] (ANT), CAS No 120-12-7
2. Benzo[a]anthracene[1,2] (BaA), CAS No 56-55-3
3. Benzo[a]anthracene[2] (BaANT), CAS No 56-55-3
4. Benzo[a]pyrene[1,2] (BaP), CAS No 50-32-8
5. Benzo[b]fluoranthene[1] (BbFA), CAS No 205-99-2
6. Benzo[e]pyrene (BeP), CAS No 192-97-2
7. Benzo[ghi]perylene[2] (BghiPER) CAS No 191-24-2
8. Benzo[j]fluoranthene (BjFA), CAS No 205-82-3

9. Benzo[k]fluoranthene[1,2] (BkFA), CAS No 207-08-9
10. Chrysen[1,2] (CHR), CAS No 218-01-9
11. Dibenzo[a,h]anthracene (DBAhA), CAS No 53-70-3
12. Fluoranthene[2] (FLU), CAS No 206-44-0
13. Phenanthrene[2] (PHE), CAS No 85-01-8
14. Pyrene[2] (PYR), CAS No 129-00-0

The hydroxylated metabolites of the PAHs are excreted in human urine both as free hydroxylated metabolites and as hydroxylated metabolites conjugated to glucuronic acid and sulfate (CDC, 2005). The analysis of urinary 1-hydroxypyrene (1-OH-PYR), the primary metabolite of pyrene, is widely used in assessment of exposure to PAHs since it is commonly found in PAH mixtures. Studies with a comparison of several biomarkers confirmed that 1-OH-PYR in urine is a valid and sensitive indicator of exposure and that periodical monitoring of 1-hydroxypyrene appears to be a powerful method in controlling occupational PAH-exposure in industries (Jongeneelen, 1997). The advantage of using 1-OH-PYR are that pyrene is present in all PAH mixtures at relatively high concentrations (2-10%) of the total PAHs, and in certain environments the pyrene content of the total PAHs is fairly constant (EC-SCF, 2002). For nonsmokers and non-occupationally exposed individuals, food accounted for 99% of the total daily pyrene intake (Van Rooij et al., 1994, cited in IPCS, 1998).

Other biomarkers are of interest when considering other PAHs, such as the 3-hydroxybenzo[a]pyrene (3-OH-BaP), metabolite of B[a]P,3-hydroyxyfluorene and 3-hydroxyphenanthrene, metabolites of fluorene and phenanthrene, respectively, and 2-hydroxynaphthalene, metabolite of naphthalene, linked to smoking, with emphasis that that the metabolite 2-hydroxynaphthalene was linked to smoking and the metabolite 1-OH-PYR was linked to dietary exposures (Nethery et al., 2012). It was also proposed that for researchers interested in predicting exposure to airborne lighter MW PAHs using urinary hydroxyfluorene and hydroxyphenanthrene metabolites be considered.

The general goal of the present assessment plan is to gather more data on human biomonitoring (HBM) of PAHs in the general and occupationally exposed populations in different countries or regions of the EU. The aim is to perform risk assessment to PAHs and to compare those calculated data with the HBM data as well as answer to the policy questions enunciated in the Scoping document for 2018, Deliverable Report D4.2 June 2017, page 117 (Tschersich et al., 2018):

- 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 practices?

2 Methodology

2.1 Literature review

A literature review was performed to find HBM data presenting PAHs exposure in general population and in workers, including the risk assessment calculations. An extensive review of existing literature was made, concerning general population, air pollution, food safety and occupational settings.

Search terms:

PAH[All Fields] AND ("polycyclic aromatic hydrocarbons"[MeSH Terms] OR ("polycyclic"[All Fields] AND "aromatic"[All Fields] AND "hydrocarbons"[All Fields]) OR "polycyclic aromatic hydrocarbons"[All Fields]) AND ("environmental monitoring"[MeSH Terms] OR ("environmental"[All Fields] AND "monitoring"[All Fields]) OR "environmental monitoring"[All Fields]) AND ("environmental monitoring"[MeSH Terms] OR ("environmental"[All Fields] AND "monitoring"[All Fields]) OR "environmental monitoring"[All Fields] OR ("biological"[All Fields] AND "monitoring"[All Fields]) OR "biological monitoring"[All Fields]) "occupational exposure"[All Fields]) OR "exposure assessment"[All Fields])

Additional filters:

“10 years”, “human” and “full text”

2.2 Risk assessment for the example populations

In following section text is divided into two parts: the first part is considering the general population risk assessment, based on inhalation and oral exposure, and the second part is considering occupational risk assessment.

2.2.1 Inhalation exposure

Since there is no unequivocal biomarker of exposure to atmospheric PAHs in environmental exposure scenarios relevant to the general population, and scarce data for some biomarkers such as 3-OH-BaP is found in literature, we have focused on 1-OH-PYR measurements in urine. In fact, as described elsewhere (RAC-Committee for Risk Assessment, 2018a) some papers confirmed good correlation between 1-OH-Pyr in urine and BaP or total PAHs in air. 1-OH-PYR is a pyrene metabolite and is therefore an indirect marker of exposure to PAH mixtures that include BaP.

Concerning general population exposure to PAHs, 9 papers were selected that presented 1-OH-PYR HBM data (Aquilina et al., 2010; Bartolomé et al., 2015; Costa et al., 2016; Franken et al., 2017; Gatti et al., 2017; Leroyer et al., 2010; Polanska et al., 2014; Schoeters et al., 2017; Urbancova et al., 2017). Among these, 3 also reported air pollution data (external exposure), that might allow the comparison of the risk assessment (RA) estimated with and without HBM.

An attempt was made to calculate excess life time risk (ELCR) for lung cancer based on dose response relationship, proposed by the ECHA-RAC (RAC-Committee for Risk Assessment, 2018b). In that document, the following dose-response equations were proposed:

$$RRx = 1 + 0.2 \times X \times 3.03/100$$

where RRx is relative risk, transformed from the occupational exposure to continuous exposure using an adjustment factor of 3.03 and X is cumulative exposure in µg BaP/m^3-years. For transforming the equations derived in ECHA-RAC document, from occupational exposure to continuous exposure of the general population over 70 years, they used an adjustment factor of 3.03[15] to account for different conditions of exposure compared to workers. Excess lifetime cancer risk (ELCR) could then be estimated for the general population using the equation:

$$ELCR = 0.07 \times (RRx-1)$$

where ELCR is excess lifetime risk for lung cancer, based on 7% lung cancer registrations in EU-28 countries (men population).

The low levels of PAHs metabolites described in urine in the studies did not allow the back-calculation of exposure levels using HBM in the RAC 2018 approach, thus no values of HBM-based ELCR concerning general population are presented in this work. Then, ELCR was estimated, based on the concentration of airborne BaP (µg/m^3) described in the papers.

As a reference for a qualitative appreciation of the values obtained, World Health Organisation (WHO) guidance suggests that the unit risk of lung cancer is 87 x 10^{-6} per ng BaP/m^3 for lifetime exposure (WHO, 2000), therefore under most regulatory programs, an ELCR of 10^{-6} or less represents virtual safety while ELCR greater than 10^{-4} represents high risk (Ambient air pollution by PAHS, (European Commission, 2001).

2.2.2 Oral (dietary) exposure

The risk assessment for general population due to dietary exposure to PAHs, based of the HBM data was not possible to estimate from the same reasons as mentioned above. In this case again the ECHA-RAC (2018) equations were used of dietary (oral) exposure to four PAHs congeners (PAH4: BaA, BbF, BaP and CHR) and to eight PAHs congeners (PAH8: PAH4 + BkF, BghiP, DBahA and IP) .

The ECHA-RAC dose-response relationships for oral exposure are based on the benchmark dose lower confidence limit ($BMDL_{10}$) derived by the European Food Safety Agency (EFSA), related to 10% response (0.1) for tumor[16] bearing animals (mice) from the 2-year oral carcinogenicity study on coal tar mixtures performed by Culp et al. (1998; referred in the EFSA, 2008). $BMDL_{10}$ for PAH4 amounted to 340 µg/kg b.w./day and $BMDL_{10}$ for PAH8 amounted to 490 µg/kg b.w./day (EFSA, 2008). In combination with an allometric factor of 7 for mice, the following ELCR could then be estimated (RAC-Committee for Risk Assessment, 2018b):

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

$$PAH8: ELCR = 0.1 \times 7/490 = 1.43 \times 10^{-3} \times \text{exposure dose per µg/kg b.w./day}$$

Where exposure dose refers to the median dietary (oral) exposure to PAH4 and PAH8, respectively, which was derived by the EFSA (2008) for mean and high consumers across the European countries. The median dietary exposure to PAH4 and PAH8 for mean dietary

[15] Adjustment factor = 20 m^3/d / 10 m^3/d × 7 d/5 d × 52 w/48 w = 3.03, according to (RAC-Committee for Risk Assessment, 2018b).
[16] Tumours of the liver, lung, forestomach, small intestine, hemangiosarcomas, histiocytic sarcomas and sarcomas of the mesentery, forestomach, skin and kidney (EFSA, 2008)

consumers amounted to 19.5 ng/kg b.w/day and to 28.8 ng/kg b.w./day, respectively. For high level dietary consumers, the median dietary exposure to PAH4 and PAH8 amounted to 34.5 ng/kg b.w./day and to 51.3 ng/kg b.w./day, respectively (EFSA, 2008). In addition the EFSA derived also the median dietary exposure for BaP which varied for mean and high dietary consumers between 3.9 ng/kg b.w./day and 6.5 ng/kg b.w./day, respectively.

Alternatively, an attempt has been made to estimate ELCR based on pyrene intake following the ECHA-RAC equations. The pyrene intake was modelled in the HBM4EU WP12.5 (Sarigiannis & Karakitsios, 2018) from the HBM data on the pyrene metabolite 1-OH-PYR. In this modeled exposure reconstruction for general population data from HBM of urinary 1-OH-Pyr across some of the EU countries (Belgium, Czech, Denmark, France, Germany, Greece, Italy) were used. According to WP12.5 report, the median intake estimates for pyrene ranges between 0.025 µg/kg b.w./day for non-smokers in Belgium to 0.240 µg/kg b.w./day for smokers in Netherlands. Median daily intake across EU countries was modeled to be around 0.05 µg/kg_b.w./day. It was also estimated in that report that dietary intake accounts for ca. 90% of total exposure to PAHs in the European general population (with exclusion of occupational exposure and smokers) (Sarigiannis & Karakitsios, 2018).

By assuming that pyrene is an indirect marker of exposure to PAHs mixtures that include BaP and that 1-OH-PYR has been linked to dietary exposures (Nethery et al., 2012) ELCR was estimated using ECHA-RAC dose-response equation.

2.2.3 Occupational exposure

Although it might be preferable to monitor total PAHs or a selection of PAHs, considering the vast and consistent amount of data presented for benzo[a]pyrene and the fact that benzo[a]pyrene is considered as one of the more potent PAH carcinogens, most of the published studies have preferred the use of benzo[a]pyrene as a marker substance for overall airborne PAH exposure for practical reasons (SCOEL, 2016). This was also the rational followed in 2018 by RAC from European Chemicals Agency in 2018.In this report. The approach described for inhalation exposure, based on RAC, was followed (RAC-Committee for Risk Assessment, 2018b). Calculations were based on 28 studies that were published reporting exposure occurring in sites based in Europe using biomonitoring tools (further details in next sections and Table 5).

3 Summary of hazard characterisation

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 (IARC - International Agency for Research on Cancer, 2010a). PAH mixtures and BaP are genotoxic carcinogens, for which safe health-based exposure limits cannot be derived for the general population. However, quantitative risk assessment procedures may be used to approximate the carcinogenic risk, dependent on the dose of PAH, by using BaP as indicator substance.

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).

In 2016, and according to previous assessments, the SCOEL (Scientific Committee on Occupational Exposure Limits) defined that a mean airborne 8h-TWA PAH exposure over 40

working years in the order of 6 ng BaP/ m^3 would lead to an excess lung cancer mortality rate of 4 x 10^{-5} (Bolt et al., 2016).

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) (Table 1).

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

4 Summary of available exposure assessment

PAHs ubiquity in the environment is a cause for concern in any case. Biomarkers of exposure most widely used for PAHs are the levels of certain urinary PAH metabolites. Those confirm that humans are exposed internally to a variety of PAHs, including those that are genotoxic/carcinogenic (http://www.chemsafetypro.com/Topics/EU/CLP_Regulation_EC_No_1272_2008.html).

The studies on PAHs exposures are far more abundant regarding external levels of PAHs. Results from several countries, like Czech Republic, Greece, Italy, Poland, Portugal, Spain and United Kingdom have been reported. For sake of clarity they are described separated by country in the following section.

4.1 Review of external exposure studies

The most abundant PAH in Czech Republic (Brno) was phenanthrene with a concentration in the air of 26.89 ± 19.11 ng/m^3 in winter 2009 and 24.04 ± 8.56 ng/m^3 in winter 2013. The summer concentrations were much lower compared to those measured in the winter. BaP varied seasonally between 0.10 ± 0.05 ng/m^3 (summer 2012) and 0.24 ± 0.18 ng/m^3

(summer 2010) and 1.35 ± 0.14 ng/m^3 (winter 2013) and 2.37 ± 1.95 ng/m^3 (winter 2012). Concerning the annual variations of BaP which is a part of legislation, the annual mean concentrations of BaP were 6.16 (2009), 6.32 (2010), 5.59 (2011), 6.21 (2012), and 2.62 ng/m^3 (2013). Between 2009 and 2012, the BaP concentration exceeded the legislation limit of 1 ng/m^3 six times. The sum of all 11 PAH concentrations annually ranged from 19.28 ± 19.02 ng/m^3 (2011) to 40.37 ± 21.35 ng/m^3 (2013). If the sum of PAHs is converted to BaP equivalent, concentration values in the range of 7.19 ± 7.48 ng/m^3 up to 27.09 ± 32.52 ng/m^3 are obtained (Bulejko et al., 2016). In another Czech Republic population (Ostrava and five rural towns in Southern Bohemia) the median concentration of BaP (7.8 ng/m3) was approximately 8-times higher than the median concentration in the background region (1.1 ng/m3) (Choi et al., 2017). Considering two more populations of Czech Republic, concentrations of BaP in summer of 2013 were 1.31 ng/m3 in Karviná and 0.44 ng/m3 in České Budějovice and in the winter of 2014 were 5.15 ng/m3 and 1.43 ng/m3, respectively (Šrám et al., 2016). With this example we can conclude that the environmental levels are highly variable seasonally and even within the same country.

In Greece, the median Σ19PAHs levels at the urban background site were 8.31, 9.82 and 9.91 ng/m3 for the $PM_{1.0}$, $PM_{2.5}$ and PM_{10} fraction, respectively, during winter 2012-13. At the traffic station, the corresponding levels were 2.82, 3.52 and 3.92 ng/m3 (Sarigiannis et al., 2015). This shows that PM/PAHs emitted from biomass burning for space heating during winter time is higher than PM/PAHs emitted from traffic sources.

In Italy, the concentrations of PAHs in the air (at the three Tuscany regions) ranged between 0.92 ng/m^3 during the warm period in Livorno and 13 ng/m^3 during the cold period at a sampling site next to a busy road in the center of Florence. BaP was detected at low concentrations and was in all cases below the maximum limit value of 1.0 ng/m^3 set by the European Union Air Quality Daughter Directive (Martellini et al., 2012). Also, in Italy, in artificial-turf playing fields of 13 different places, the concentrations of BaP detected were 0.98 mg/kg (dry weight) approximately 9 times higher than the soil contamination standards in Italy (0.1 mg/kg). The same study compared these values with the concentrations of BaP in the air during the use of these playing fields and determined 0.05 ng/m3 (fields and background air) and 0.09 (urban air) ng/m3 (Menichini et al., 2011).

Regarding Portugal, in Lisbon the sum concentration of 16 PAHs of USEPA list was 1544 μg/kg of surface soils while in Viseu it was 169 μg/kg (Cachada et al., 2012). This discrepancy between both cities might be for the lack of industrial activity in Viseu whereas in the greater area of Lisbon there are an excess of transports traffic and industries. The major sources of PAHs in Viseu might be agriculture practices, forest fires, house heating and traffic (Cachada et al., 2012). In another study made in Portugal, Estarreja, the median sum of 16 USEPA PAHs was 72 μg/kg (urban) and 111 μg/kg (agricultural). Also, in Portugal, but now a study from the indoor atmosphere of a preschool from Porto, showed values of PAHs concentration of 6.89 ng/m^3 (sum of 16 USEPA PAHS) for $PM_{2.5}$ and 5.4 ng/m^3 for PM_1. In the surrounding outdoor atmosphere, the same authors measured the sum of PAHs for $PM_{2.5}$ of 7.73 ng/m^3. Although slightly higher outdoors, the concentration of PAHs bound to $PM_{2.5}$ was not statistically different (Oliveira et al., 2015). Slezakova et al. performed a similar study but now comparing also the indoor atmospheric PAH values between preschools and elementary schools (Slezakova et al., 2017). Curiously, the authors showed that the indoor levels of ΣPAHs at preschools were significantly different from those observed at elementary schools. The sum of 16 PAHs values in indoor air ranged from 0.7 ng/m3 (preschool 1) to 23.6 ng/m3 (elementary school 3), whereas the mean concentrations were between 4.2 ng/m3 (preschool 2) and 16.4 ng/m3 (elementary school 3). Also, from Porto, Slezakova et

al. determined PAHs level ranging from 16.8 to 149 ng/m3, with a mean of 70 ng/m^3 and phenantrene being the most observed PAH (Slezakova et al., 2013). In an additional study from Porto comparing smoking and non-smoking populations, Slezakova et al. showed that mean concentrations of PAHs at smoking homes were 17.1 ng/m3 in PM10 and 16.6 ng/m3 in PM2.5, while in non-smoking houses were 7.60 ng/m3 in PM10 and 7.16 ng/m3 in PM2.5 (Slezakova et al., 2014).

Regarding Spain, Callén et al. showed that in the Zaragoza the dominant PAH in atmosphere were those with four and five rings (Chry, Fth, BbF, Py and BghiP) that were measured at the highest average concentrations (Callén et al., 2014). The concentration of the sum of all PAHs determined in the air was 2.14 ng/m^3 regardless the season and 1.31 ng/m^3 in the warmer season and 2.84 ng/m^3 in the colder season. In another region of Spain, Terragona, the highest concentrations detected were of PA, Ant, AcPy and Flu in the gas phase of air samples (24.5–12.8 ng/m^3) and semi-volatile PAHs as FluT in total suspended particle phase (3.8 ng/m^3). The mean values of the sum of the PAHs ranged between 16.6 ng/m3 (site 1) and 34.8 ng/m^3 (site 2) (Cuadras et al., 2016)(Cuadras et al., 2016). In 2016, in Toledo, a tire landfill fire occurred and the mean concentration of the sum of the 16 USEPA PAHs was 134 ng/m3 for the adjacent resident area of the landfill, and 19.5 ng/m3 and 22.7 ng/m3 for residential areas far from the tire landfill (Nadal et al., 2016).

In United Kingdom a study comparing indoor and outdoor air BaP concentration, detected that mean concentrations were generally lower indoors (BaP=0.10 ng/m3) than outdoors (BaP=0.19 ng/m3), apart from indoor environments with wood burners (BaP=2.4 ng/m3) or Environmental Tobacco Smoke (BaP=0.6 ng/m3) (Delgado-Saborit et al., 2011).

The EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) calculated the median dietary exposure across European countries (EFSA, 2008). These varied for mean and high dietary consumers alone between 3.9 ng/kg b.w./day and 6.5 ng/kg b.w./day, respectively for BaP; between 10.7 ng/kg b.w./day and 18.0 ng/kg b.w./day, respectively for PAH2; between 19.5 ng/kg b.w./day and 34.5 ng/kg b.w./day, respectively for PAH4 and between 28.8 ng/kg b.w./day and 51.3 ng/kg b.w./day, respectively for PAH8. The two highest contributors to the dietary exposure were cereals and cereal products, and sea food and sea food products.

4.2 Limit values of PAHs in different segments, based on RA

The limits established in EU are mostly external exposure limits (consumer goods, air, soil, food and water) and are some are summarized in Table 2.

Regarding occupational exposures there are different Occupational Exposure Limits (OELs) for PAHs mixture in several European Countries. Even for United Sates the OELs are different from the ones reported in Europe. For instance, for a Threshold Limit Value (TLV) of 8 hours of exposure (TLV-Time-Weighted Average), the values varied between 0.05 mg/m3 and 0.2 mg/m^3. On the other hand, in Germany there is no OEL because PAH is classified as carcinogenic.

Table 2: Limit values of PAHs in different matrices as currently applied (based on RA)

Regulation	Source	Type of product/ material	Limit Value allowed	PAHs
Regulation (EU) No.1272/2013 to amend Entry 50 of Annex XVII to REACH Regulation (EC) No.1907/2006	Rubber and plastic	Consumer goods - rubber and plastic products	1 mg/kg	PAH8: Benzo[a]pyrene, Benzo[e]pyrene, Benzo[a]anthracene, Chrysen, Benzo[b]fluoranthene, Benzo[j]fluoranthene, Benzo[k]fluoranthene and Dibenzo[a,h]anthracene
Regulation (EU) No.1272/2013 to amend Entry 50 of Annex XVII to REACH Regulation (EC) No.1907/2006	Rubber and plastic	Consumer goods – toys and baby toys	0.5 mg/kg	PAH8: Benzo[a]pyrene, Benzo[e]pyrene, Benzo[a]anthracene, Chrysen, Benzo[b]fluoranthene, Benzo[j]fluoranthene, Benzo[k]fluoranthene and Dibenzo[a,h]anthracene
Regulation (EC) No 1881/2006	Food	Oils and fats (excluding cocoa butter and coconut oil) intended for direct human consumption or use as an ingredient in food	2.0/10.0 µg/kg	Benzo[a]pyrene/PAH4 (PAH4: Sum of Benzo[a]pyrene, Benzo[a]anthracene, Benzo[b]fluoranthene and Chrysene)
Regulation (EC) No 1881/2006	Food	Cocoa beans and derived products	5.0/30.0 µg/kg fat	Benzo[a]pyrene/PAH4
Regulation (EC) No 1881/2006	Food	Several Ingredients and foods	1.0 up to 50.0 µg/kg	Benzo[a]pyrene/PAH4
Occupational Exposure Limits (OELs) for PAHs mixture (Directive 2017/2398, European Parliament, 12 of December)	Air		0.05 mg/m^3 and 0.2 mg/m^3	PAH mixtures
German legislation	Air		No limit since it's a carcinogenic mixture	PAH mixtures

4.3 Current biological guidance (reference) values

It was reported in the EFSA document (2002) that the background concentrations of 1-OH-PYR in urine of persons from different countries range from 0.06 to 0.23 µmol/mol creatinine (Kang et al., 1995, cited in EFSA, 2002).

In 2001 Jongeneelen made an attempt to propose a three-level benchmark guideline for urinary 1-OH-PYR. i.e. the reference value as a 95th percentile in non-occupational exposed controls is 0.24 µmol/mol creatinine and 0.76 µmol/mol creatinine for non-smokers and smokers, respectively. This was set as the first level of the benchmark guideline. A no-biological-effect-level of 1-OH-PYR in urine of exposed workers was found at 1.4 µmol/mol creatinine and was the lowest reported level at which no genotoxic effects were found and therefore the estimate for the second level of the benchmark guideline.

In two types of industry, coke ovens and primary aluminium production, the regression of airborne PAH concentrations and urinary 1-OH-PYR concentrations in exposed workers has been also studied. The correlation of airborne concentrations and urinary 1-OH-PYR in urine of workers from coke ovens and in the primary aluminium industry was used to estimate the level of urinary 1-OH-PYR equal to the present occupational exposure limit (OEL) of PAH. The concentration of 1-OH-PYR in urine equal to the OEL is 2.3 µmol/mol creatinine and 4.9 µmol/mol creatinine, respectively, in these two industries. These latter values presented the third level of the benchmark guideline (Jongeneelen, 2001).

According to Wilhelm et al. (2008) the reference value for 1-OH-PYR in urine was derived from the representative adult population data collection of the 1998 German Environmental Survey (GerES III) and the representative data collection for children of the German Environmental Survey on Children, 2003/06 (GerES IV). For the non-smoking general population (aged 3–69 years) the reference value for 1-OH-PYR in urine is 0.5 mg/L (corresponding to 0.3 mg/g creatinine). Additionally, the background exposure levels were derived for 1-naphtol and 2-naphthol in urine for adult non-smokers at <30 mg/L and <20 mg/L, respectively (Schulz et al., 2011).

According to the Società italiana Valori di Riferimento (SIVR), 2011, reference values for 1-OH-PYR for the Italian population are 0.3 µg/L (non-smokers) and 1.0.7 µg/L (smokers) (SIVR, 2011).

In addition to the review done by the Scientific Committee on Occupational Exposure Limits (SCOEL), in 2016 there are no Biological Limit Values (BLVs) for BaP or for PAH mixtures containing BaP available to date (Bolt et al., 2016). There is however a BAR (Biologischer Arbeitsstoff-Referenzwert / biological reference value) by the German Research Foundation (Deutsche Forschungsgemeinschaft, DFG) of 0.3 µg 1-OH-PYR/g creatinine in urine. The UK has a biological monitoring guidance value of 4 µmol 1-OHPYR/mol creatinine (approx. 8 µg 1-OH-PYR/g creatinine) based on the 90th percentile value of a survey of workplaces with exposure to PAH(Unwin et al., 2006). The ACGIH have proposed a Biological Exposure Index (BEI) of 2.5 µg/l (adjusted for the pyrene and BaP ratio of the PAH mixture to which workers are exposed) (ACGIH 2016). Available biological guidance and reference values are summarized in Table 3.

Table 3: Available biological reference/guidance values.

Reference	Population	PAH/OH-PAH	Level
SIVR 2011	General	1-OH-PYR	<0.5 µg/L of urine in non-smokers[1] <1 µg/L in smokers[1]
German Federal Environment Agency 2008	General (aged 3–69 years)	1-OH-PYR	<0.5 µg/L of urine[1]
UK biological monitoring guidance value	Workplaces	1-OH-PYR	4µmol/mol creatinine
ACGIH*	Workplaces	PYR/BaP ratio	2.5 µg/L of urine

[1]Reference values

*American Conference of Governmental Industrial Hygienists

5 Risk characterisation and uncertainty analysis

5.1 General population

The RAC dose response relationship for lung cancer was used to calculate ELCR for the cumulative continuous exposure of the general population (4 h for 70 years), by using the lifetime risk of contracting lung cancer from the EU-28 registrations. As it is evident from the RAC equation, using the unit relative risk value (URR) of 1.20, one year's exposure of 1 ng/m^3 adjusted over 70 years will therefore lead to a lifetime excess lung cancer risk of 3.0×10^{-5}. The URRs were derived based on meta-analysis of 39 epidemiological studies on occupational exposures to PAH mixtures, that were critically evaluated before inclusion in a meta-analysis, based on nearly 3000 cases. Due to limited exposure data in the underlying epidemiological studies, the mean URR for lung cancer at 100 µg BaP/m^3 years of 1.20 (95% CI: 1.11 – 1.29) derived by Armstrong et al. (2003, 2004) from all 39 underlying cohort studies was used in the carcinogenicity risk assessment for CTPHT. This average value across all industries analysed is recommended for prediction of cancer risk associated with exposures to CTPHT and its volatiles CTPVs (SCOEL 2016; TNO/RIVM 2008). Since the exposure routes and composition of the PAHs mixtures will differ from that in the occupational settings that serve as a basis to derive a dose-response, the use of this dose-response relationship for the general population results in considerable uncertainties to the estimates of excess lung cancer risk for the general population.

With regard to the RAC dose response relationship for cancers via oral route, estimation of ELCR based on the $BMDL_{10}$ values for PAH4 and PAH8 (derived by the previously mentioned EFSA study, 2008) may be given preference since the relationship based on BaP exposure only does not account for carcinogenicity of other PAHs to which humans are exposed via the food. Based on the available data relating to occurrence and toxicity of PAHs in foods, the EFSA suggested that a combination of 4 PAH4 and PAH8 are more suitable indicators for food contamination than BaP alone, with emphasis that PAH8 not provided much added value compared to PAH4 (EFSA, 2008).

5.2 Occupational exposure

As mentioned previously, the risk characterization done by most of the agencies and stakeholders are based on BaP data. However, for some occupational settings, BaP might not be suitable indicator for PAHs exposure, particularly if used alone. Indeed, there are occupational settings scenarios where elevated levels of lower ring number PAHs may be present, such as naphthalene, anthracene and phenanthrene. Additionally, where the mixture of PAH content is unknown, exposure to the 16 priority PAHs should be assessed in the first instance.

ECHA-RAC (2018) used the relationship between urinary 1-OH-PYR and BaP in air for selected occupational settings to back-calculate the exposure levels (concentration of airborne BaP) in $\mu g/m^3$. One limitation of this relationship is that it is not accurate in the low exposure range and cannot estimate exposure levels with urinary 1-OH-PYR values below 1.13 µmol/mol (it would be negative below the corresponding air concentration). That would be the case of most of the levels reported in the general population, using HBM.

In HBM4EU, under WP12.5, focus is on how to derive intake estimates corresponding to the HBM (aggregated) data available for the 1st set of priority compounds that have been identified as such in the HBM4EU context, including PAHs. The optimized methodology for exposure reconstruction already described in AD12.6 was applied for this purpose. Regarding their findings, intake levels of pyrene resulted in a cancer risk significantly lower than 10^{-6}; however, the lack of BaP specific biomarker data was a problem for estimating intake levels of the compound that is driving PAHs mixture carcinogenicity. All these estimates include coarse assumptions and extrapolations, however at this stage these estimates can be used for characterizing the potential risk from general population exposure to PAHs.

6 Improving the RA by biomonitoring

6.1 Evaluation of the available biomonitoring data

An inventory of HBM programmes worldwide was recently published by Choi et al. (2015) showing 11 studies focusing on PAHs in the general population. Selected HBM studies in the EU countries since 2010 regarding general population are listed in Table 4 and occupational population in Table 5. In the following section we summarized in brief some of available PAHs HBM studies.

The significance of diet as an exposure route for PAHs was studied in one of the first studies of this kind by Buckley and Lioy (1992). The associated kinetics of urinary 1-OH-PYR elimination were examined through a controlled human exposure study. Results showed that a 100 to 250-fold increase in a dietary BaP dose paralleled a four to 12-fold increase in urinary 1-OH-PYR elimination. Mean elimination rates during minimal exposure periods ranged from 6 to 17 ng/h whereas peak elimination rates of 60 to 189 ng/h were seen after a meal high in PAHs. A biexponential model fitted to a limited number of urinary 1-OH-PYR elimination points gave mean kinetic parameter estimates for t1/2 of 4.4 hours and tmax of 6.3 hours. It was concluded that dietary exposure to PAHs is potentially as substantial as some occupational exposures and therefore requires consideration in studies of exposure to PAHs. The dietary control strategies and the kinetic parameters defined in this investigation provided data for the control of this exposure route when examining other sources of exposure.

The Centers for Disease Control and Prevention's National Health and Nutrition Examination Survey (NHANES) uses OH-PAHs to establish reference range concentrations for the US population, and to set benchmarks for future epidemiologic and biomonitoring studies (Li et al., 2008). For the years 2001 and 2002, 22 OH-PAH metabolites were measured in urine specimens

from 2748 NHANES participants. Percentages of samples with detectable levels ranged from nearly 100% for metabolites of naphthalene, fluorene, phenanthrene, and pyrene, to less than 5% for metabolites from parent compounds with higher molecular weight such as chrysene, benzo[c]phenanthrene, and benz[a]anthracene. The geometric mean for 1-OH-PYR was 49.6 ng/L urine, or 46.4 ng/g creatinine. Children (ages 6-11) generally had higher levels than did adolescents (ages 12-19) or adults (ages 20 and older). Model-adjusted, least-square geometric means for 1-PYR were 87, 53 and 43 ng/L for children, adolescents (ages 12-19) and adults (ages 20 years and older), respectively. Log-transformed concentrations for major detectable OH-PAHs were significantly correlated with each other. The correlation coefficients between 1-OH-PYR and other metabolites ranging from 0.17 to 0.63 support the use of 1-OH-PYR as a useful surrogate representing PAH exposure (Li et al., 2008).

Li et al. (2010) studied non-occupational inhalation and ingestion exposure to PAHs in 8 non-smoking volunteers through personal air sampling and urinary biomonitoring. The study period was divided into 4 segments (2 days/segment), including weekdays with regular commute and weekends with limited traffic related exposures; each segment had a high or low PAH diet. Personal air samples were collected continuously from the subjects while at home, at work, and while commuting to and from work. All urine excretions were collected as individual samples during the study. In personal air samples, 28 PAHs were measured, and in urine samples 9 OH-PAHs from 4 parent PAHs (naphthalene, fluorene, phenanthrene and pyrene) were measured. Naphthalene was found at higher concentrations in air samples collected at the subjects' residences, whereas PAHs with four or more aromatic rings were found at higher levels in samples taken while commuting. Urinary OH-PAH biomarker levels increased following reported high inhalation and/or dietary exposure. On days with a low PAH diet, the total amount of inhaled naphthalene during each 24-hour period was well correlated with the amount of excreted naphthols, as was, to a lesser extent, fluorene with its urinary metabolites. During days with a high dietary intake, only naphthalene was significantly correlated with its excreted metabolite. These findings suggest that this group of non-occupational subjects were exposed to naphthalene primarily through indoor air inhalation, and exposed to other PAHs such as pyrene mainly through ingestion.

In the work of Leroyer et al. (2010) they studied two groups of 15 and 10 non-smoking, healthy men and women, exposed for approximately 6h to ambient air at two outdoor locations close to metallurgical industries, and at one indoor location in an urban setting. Atmospheric measurements of 16 "priority" PAHs were carried out during each exposure. Urinary 1-OH-PYR and 3-OH-B[a]P were also analysed, samples being taken the morning before exposure, at the end of exposure, then 4 and 15 h after the end of exposure. Concentrations of 1-OH-PYR were not correlated with atmospheric concentrations of PAHs to which subjects were exposed, nor with the concentrations of 3-OH-B[a]P. Nearly 80% of measurements of 3-OH-B[a]P were lower than the LOD and no relationship between atmospheric concentrations and urinary metabolites was observable. However, the percentage of post-exposure values of 3-OH-B[a]P greater than the LOD increased significantly with the median of atmospheric concentrations of PYR, B[a]P and ΣPAH at the different sites (test of linear trend, $p<0.02$ in all cases). They concluded that neither 1-OH-PYR nor 3-OH-B[a]P were an unequivocal biomarker of exposure to atmospheric PAHs in environmental exposure scenarios relevant to the general population and that it would be interesting to investigate other urinary monohydroxy PAH metabolites in this context.

In their study, Li et al. (2010) found that urinary OH-PAHs biomarker levels increased following reported high inhalation and/or dietary exposure to PAHs. They studied the non-occupational inhalation and ingestion exposure to PAHs in 8 non-smoking volunteers through personal air sampling and urinary PAHs biomonitoring, taking into account traffic related exposures as well as

dietary exposure (a high or low PAH diet). They found that on days with a low PAHs diet, the total amount of inhaled naphthalene during each 24-hour period was well correlated with the amount of excreted naphthols, as was, to a lesser extent, fluorene with its urinary metabolites. During days with a high dietary intake, only naphthalene was significantly correlated with its excreted metabolite. These findings suggested that the studied group of non-occupational subjects were exposed to naphthalene primarily through indoor air inhalation, and exposed to other PAHs such as pyrene mainly through ingestion.

Recently, Nethery et al. (2012) studied exposure to PAHs by collecting air and urine samples among pregnant women pre-selected as living in "high" and “low” exposure areas. They analyzed first-morning urine voids from all 3 trimesters of pregnancy for urinary PAH metabolites and compared these to personal air PAH/PM2.5/NO2/NOx samples collected in the 3rd trimester of pregnancy. They also evaluated activities and home characteristics, geographic indicators and outdoor central site PM2.5/NO2/NOx samples (all trimesters). Personal air exposures to the lighter molecular weight (MW) PAHs were linked to indoor sources (candles and incense), whereas the heavier PAHs were related to outdoor sources. Geometric means of all personal air measurements were higher in the "high" exposure group. Urine metabolites were only directly correlated with their parent air PAHs for phenanthrene and fluorene and that specific metabolites (3-hydroxyphenanthrene and 3-hydroyxyfluorene) may be related to their parent air PAH exposures. The metabolite 2-hydroxynaphthalene was linked to smoking and the metabolite 1-OH-PYR was linked to dietary exposures. They suggested that hydroxyfluorene and hydroxyphenanthrene metabolites be considered for predicting exposure to airborne lighter MW PAHs using urinary PAHs metabolites, and that centrally monitored heavier MW PAHs could be used to predict personal exposures for heavier PAHs.

In their work, Ranzi et al. (2013) described a human biomonitoring cross-sectional pilot study in Modena, Italy as a part of the authorization process for the solid waste incinerator (SWI). Between May and June 2010, 65 subjects living and working within 4km of the incinerator (exposed) and 103 subjects living and working outside this area (unexposed) were enrolled in the study. Among different substances also urinary PAHs were analysed. Information about lifestyle, anthropometric characteristics, residence, and health status was collected by a self-administered questionnaire. Exposure to particulate matter (PM) emitted from the SWI was estimated using fall-out maps from a quasi-Gaussian dispersion model. Urinary PAHs were higher in exposed than in unexposed subjects for phenanthrene, anthracene, and pyrene (median levels: 9.5 vs. 7.2 ng/L, 0.8 vs. <0.5 ng/L and 1.6 vs. 1.3 ng/L, respectively, $p<0.05$). Multiple linear regression analysis showed that urinary fluorene, phenanthrene, anthracene and pyrene were inversely correlated to the distance of a subject's residence from the SWI. Besides this, urinary fluorene and phenanthrene were directly correlated to PM exposure.

The aim of the work of Campo et al. (2014) was to assess exposure to carcinogenic PAHs, to evaluate the role of occupational and environmental variables on PAHs levels, and to compare present results obtained by an application of a new solid phase microextraction-gas chromatography-mass spectrometry method (SPME-GC-MS) with those previously obtained with a less sensitive method. The urinary unmetabolized 3-, 6-ring PAHs were studied in a total of 104 coke oven workers (CW) from Poland [recruited in 2000 (CW-2000; n=55) and 2006 (CW-2006; n=49)], and 45 control subjects from the same area, provided urine spot samples for measurement of 10 PAHs (from phenanthrene to benzo[g,h,i]perylene). The comparison between the two methods was performed only on CW-2000 subjects. Information regarding personal characteristics and job variables was collected by a questionnaire. The new method applied enabled quantification of 5-, 6-ring PAHs. Chrysene and benz[a]anthracene were the most abundant carcinogenic PAHs with median levels of 43.4, 13.4, and 2.3 ng/L and 45.9, 14.9, and 0.7 ng/L in CW-2000, CW-2006,

and controls, respectively, while benzo[a]pyrene levels were 6.5, 0.7 and <0.5 ng/L. The multiple linear regression model showed that the determinants of exposure were the use of wood and/or coke for house heating for controls, and job title or the plant for CW-2006. The obtained results showed that urinary PAHs can discriminate exposure at different levels. Moreover, the simultaneous determination of several PAHs allows for the development of excretion profiles to assess exposure to specific compounds.

Polanska et al. (2014) studied the impact of PAHs exposure on various anthropometric measures of birth outcomes. The study population consisted of 210 nonsmoking pregnant women. Urine samples collected between 20th and 24th week of pregnancy were used for analysis of the 1-, 2-, 3-, 4-, and 9-OH-PHE, 1-OH-PYR, 1,6 + 1,8-DI-OH-PYR, PHE-1,2-diol, and PHE-9,10-diol by gas chromatography-mass spectrometry (GC-MS). Environmental tobacco smoke exposure (ETS) was assessed by cotinine level in saliva using a stable isotope dilution LC-ESI-MS/MS method. The mean PAHs metabolite concentrations were in the range of 0.15 µg/g creatinine for 9-OH-PHE to 5.9 µg/g creatinine for PHE-9,10-diol. It was shown that none of the individual PAH exposure markers demonstrate a statistically significant influence on birth outcomes. Interestingly a statistically significant association was found between the sum of OH-PHE along with cotinine level and the cephalization index after adjusting for potential confounders (P = 0.04). This study provides evidence that combined exposure of pregnant women to common environmental pollutants such as PAHs and ETS might adversely affect fetal development. Thus, reduction of human exposure to these mixtures of hazardous compounds would in particular result in substantial health benefits for newborns.

Bartolome et al. (2015) reported on the nationwide Human Biomonitoring survey on PAH exposure performed in Spanish adults, the BIOAMBIENT.ES. The urinary metabolites 1-OH-PYR, 1-,2-,3-,4- and 9-OH-PHE and 3-OH-B[a]PYR were selected as indicators of PAH exposure. Urine samples from 957 subjects (16–65 years old) were collected during year 2009–2010. Geometric mean and 95th percentile for 1-OH-PYR in µg/g creatinine were 0.117 (non-smoker: 0.079, smokers: 0.184) and 0.67µg/g creatinine (non-smokers: 0.31, smokers: 0.69) respectively. GM and 95th percentile for sum of OH-PHEs in µg/g creatinine were 0.130 (non-smokers: 0.089, smokers: 0.317) and 1.29 (non-smokers: 0.71, smokers: 1.51) respectively. 3-OH-B[a]PYR was below the limit of quantitation (0.05µg/L) in all cases. Significant differences (p<0.05) regarding smokers and non-smokers, coal and wood heating, body mass index and second hand smoke were found, while other variables like gender, age, or diet showed no significant association. The geographical distribution of the metabolites showed higher levels in people who lived in the north and northwest of Spain. The PAH metabolites levels found were in the same range or lower than those reported from other European countries and they were higher than those found in the U.S. This study represented the first nationwide survey of exposure to PAHs in Spain and provided a background reference range for exposure to PAHs in the Spanish adult population.

In their study Thai et al. (2015) reported urinary concentrations of PAH metabolites in a small group of residents in Brisbane, Australia, and Hanoi, Vietnam, and those travelling between the two cities. Their goals were to assess a) the exposure to PAHs in the two cities through biomonitoring of urinary OH-PAH concentrations; b) the change of OH-PAH profile when people travelled between the two cities; and c) the influence of age on the concentrations of OH-PAHs. Studied group represented 16 healthy volunteers (9 adults and 7 children) from 5 families. Three families lived in metropolitan Brisbane and two families lived in metropolitan Hanoi. Their homes were not close to any heavy emission source or heavy traffic (at least 1 km away from heavy traffic). During the study, one family in Brisbane (two adults and two children) travelled to Hanoi, and then back to Brisbane. All participants were of Vietnamese origin, i.e. there was no race difference that could significantly affect the metabolism of PAHs. The adults were aged between 28

and 35 years and the children aged between 2 and 8 years. A total of 312 urine samples were collected during the study period. The first-morning urine voids were collected twice a week (Tuesday and Friday) for 10 weeks in August and September 2011. The travelling family collected additional samples for one week in July 2011 and samples before and around 6 hours after their flights. There were occasions when the participants missed the sampling date and no sample was collected. Concentrations of the urinary OH-PAHs were 3-10 times higher in participants from Hanoi than those from Brisbane. For example, the median concentrations of 1-OH-PYR was 292 pg/mL in Hanoi, compared to 64 pg/mL in Brisbane. For participants travelling from Brisbane to Hanoi and back, differences in exposure to PAHs in these two cities resulted in corresponding changes of urinary OH-PAH concentrations, demonstrating that the more polluted environment in Hanoi was likely the source for higher PAH exposure there.

The aim of study of Thai et al. (2016) was to provide the first assessment of exposure to PAHs in a large sample of the population in Queensland, Australia including exposure to infant (0–4 years). De-identified urine specimens, obtained from a pathology laboratory, were stratified by age and sex, and pooled (n=24 pools of 100) and OH-PAHs were measured by gas chromatography–isotope dilution–tandem mass spectrometry. Geometric mean (GM) concentrations ranged from 30 ng/L (4-hydroxyphenanthrene) to 9221 ng/L (1-naphthol). GM of 1-OH-PYR, the most commonly used PAH exposure biomarker, was 142 ng/L. For each metabolite the range of concentrations among age groups was relatively small, with the ratio of maximum to minimum concentrations ranging from 4 to 8, except for 1-NAP for which concentrations varied widely (924 to 375,182 ng/L). The concentration of 1-NAP in two pooled samples (Pools 15 and 19, Table 1) was two orders of magnitude higher than the GM (9221 ng/L). The highest concentrations of 1-NAP were detected in the 45–59 years age strata (7900–375,000 ng/L. The concentrations of OH-PAHs found in this study were found consistent with those in developed countries and lower than those in developing countries. They observed no association between sex and OH-PAHs concentrations. However, they observed lower urinary concentrations of all OH-PAHs in samples from infants (0–4 years), children (5–14 years) and the elderly (>60 year old) compared with samples from other age groups (15–29, 30–44 and 45–59 years) which may be attributed to age-dependent behavior-specific exposure sources.

In a study of Zajac et al. (2017) performed in Poland, they compared urinary 1-OH-PYR concentration among 647 coke plant workers (before and after working week) and among 206 non-exposed individuals from the same area, taking smoking status into consideration. Average urinary 1-OH-PYR concentration of samples collected before the working week was: 1.07 µg/g; after the working week: 2.36 µg/g and for control: 0.74 µg/g. The samples collected at the beginning of the working week were not suitable for assessment of the workers' background (non-occupational) exposition. Smoking cigarettes induced a rise in urinary 1-OH-PYR level by 16%, on average (CI: 5% – 28%), and working for a whole working week at the coke plant made urinary 1-OH-PYR levels, on average, 3.21 times higher (CI: 2.91 – 3.54). They concluded that Smoking remains a significant source of PAHs exposure, despite the fact that occupational exposure is greater.

Urbancova et al. (2017) studied OH-PAHs in 531 urine samples, collected from mothers and their newborns from two localities of the Czech Republic – heavily air polluted Karvina and control locality of Ceske Budejovice and in two sampling rounds – August–October 2013 (summer, less air polluted season) and January–April 2014 (winter, more air polluted season). From target metabolites 2-OH-NAP was the most abundant compound found in 100% of the samples. 1-OHPHEN was another frequently detected analyte (97% of samples) followed by 2-OH-PHEN (96% of samples), 2-OH-FLUO (91% of samples), 9-OH-PHEN (85% of samples), 3-OH-PHEN (81% of samples), 4-OH-PHEN (79% of samples), 1-OH-PYR (75% of samples) and 1-OHNAP (74% of samples). The concentration of all monitored OH-PAHs (expressed as the ΣOH-PAHs)

ranged from 650 to 78,000 ng/g creatinine (1.7–92 ng/mL urine). The concentrations of the most abundant 2-OH-NAP varied from 360 up to 41,000 ng/g creatinine (median 4300 ng/g creatinine, 3.3 ng/mL urine). The concentrations of other quantified analytes were about ten times lower. The authors reported that domination of 2-OH-NAP among other measured OH-PAHs was also found in other similar studies. As 1-OH-PYR, which was suggested in many studies as the most suitable exposure biomarker, was found in only 77% of the samples, although other PAHsmetaboliteswere present, they assumed that this clearly documents that a set of PAHs metabolites has to be measured to obtain more accurate data for the risk assessment process.

A cross-sectional biomonitoring study was carried out by Gatti et al. (2017) again in Modena, Italy to investigate exposure to the solid waste incinerator (SWI) emissions in relation to the body burden of selected biomarkers in the population living around the plant. Approximately 500 people, aged 18-69 years, living within 4 km from the incinerator were randomly selected form the population register. Exposure was measured through fall-out maps of particulate matter (PM), used as tracer for incinerator emissions. Besides toxic metals, ten metabolized PAHs, from naphthalene to chrysene, including 1-OH-PYR were measured in spot urine samples. Confounders, such as diet, smoking, traffic, occupation and personal characteristics were assessed by questionnaires and objective measurements, and included into multivariate linear regression models. Most abundant PAHs were naphthalene (median 26.2 ng/L) and phenanthrene (7.4 ng/L). All PAHs, but benz[a]anthracene and 1-OH-PYR were found in more than 52% of samples, and included in regression models. Significant associations between urinary PAHs and exposure were found, strong for fluorene, and weaker for naphthalene, fluoranthene and pyrene. Results were confirmed by sensitivity analyses. Correlation with variables reported in literature were observed. The study indicated that the emissions were very low and highlighted that specific urinary PAHs provided useful information about the internal dose arising from incinerator emission.

In study of Dobraca et al. (2018) performed in Northern California, USA, urinary concentrations of ten PAHs metabolites and cotinine were quantified in 431 girls age 6-8 years at baseline. Characteristics obtained from parental interview, physical exam, and linked traffic data were examined as predictors of PAHs metabolite concentrations using multivariable linear regression. Eight PAHs metabolites were detected in ≥ 95% of the girls. The most consistent predictors of PAHs biomarker concentrations were cotinine concentration, grilled food consumption, and region of residence, with some variation by demographics and season. After adjustment, select PAHs metabolite concentrations were higher for Hispanic and Asian girls, and lower among black girls; 2-naphthol concentrations were higher in girls from lower income households. Other than 1-naphthol, there was modest reproducibility over time (ICCs between 0.18 and 0.49) and the concentration from a single spot sample was able to reliably rank exposure into quartiles consistent with the multi-year average. These results confirm diet and environmental tobacco smoke exposure as the main sources of PAHs. Controlling for these sources, differences in concentrations still existed by race for specific PAHs metabolites and by income for 2-naphthol. The modest temporal variability implies adequate exposure assignment using concentrations from a single sample to define a multi-year exposure timeframe for epidemiologic exposure-response studies.

Urinary monohydroxy metabolites (OH-PAHs) of 20 PAHs were assessed in 218 three-year old children performed by Sochacka-Tatara et al. (2018) in Poland. Only 10 of all analysed OH-PAHs were present in nearly all the samples: monohydroxy metabolites of naphthalene, fluorene, phenantrene and pyrene. Of the metabolites analyzed, hydroxynaphthalenes were predominant and constituted almost 73% of total excreted OH-PAHs, while 1-OH-PYR was the least abundant (2.3% of total OH-PAHs). All measured urinary OH-PAHs were statistically significantly correlated with each other (R = 0.165-0.880) but the highest correlation coefficients with other individual OH-PAHs and with total OH-PAHs were observed for 2-OH-FLUOR. Children exposed at home to

environmental tobacco smoke (ETS) had higher concentrations of fluorene and pyrene urinary metabolites compared to those without ETS exposure; and those exposed to gas-based appliances used for cooking or heating water had higher levels of fluorene and phenanthrene metabolites than children not exposed. The use of coal, wood or oil for heating was associated with elevated levels of 1-OH-PYR. Urinary PAHs metabolites only modestly reflect high molecular weight carcinogenic PAHs exposures such as those monitored in air in the present study. None of the measured PAHs metabolites was correlated with airborne PM2.5 and only two were slightly correlated with measured higher molecular mass airborne PAHs. The average concentrations of these specific metabolites in Polish children were much higher than observed in other pediatric populations living in developed countries. These findings suggested that to capture various sources of PAHs, in addition to 1-OH-PYR, biomonitoring of PAHs exposure should include 2-OH-NAP and 2-OH-FLUOR.

6.1.1 General population

HBM studies on PAHs in general populations are scarce in the EU (biomonitoring studies with effect biomarkers are even more scarce). The available studies from literature search are summarized in Table 4. In that Table, the biomonitoring levels of PAHs and some parent PAHs in Europe (1-OH-PYR) and PYR) are shown.

Table 4: List of PAH/OH-PAH levels measured in different studies from EU countries

Country/ Study	Population	PAH/OH-PAH	Level	Reference
Belgium FLEHS	Adolescents [a]	1-OH-PYR	208.33 ng/L	(Schoeters et al., 2017)
Belgium FLEHS I	General Population	1-OH-PYR	123 ng/L	
Belgium FLEHS II	General Population	1-OH-PYR	140 ng/L	
Belgium FLEHS III	General Population	1-OH-PYR	122 ng/L	
Belgium FLEHS III	Adolescents	1-OH-PYR	180 ng/L	
Belgium FLEHS III	Adolescents	PYR, BaP	0.57 ng/L, 0.21 ng/L	
France	General Population [b]	1-OH-PYR 3-OH-BaP	T1 - 39.8 nmol/mol creatinine T2 - 41.5 nmol/mol creatinine T3 - 38.8 nmol/mol creatinine T4 - 39.9 nmol/mol creatinine < LOD	(Leroyer et al., 2010)
Italy	General Population	1-OH-PYR	50 ng/L	(Gatti et al., 2017)
Italy	General Population [c]	NAP ACY ACE FLU PHE PYR ANT FLT BaA CHR	Exposed 48.1 Unexposed 45.7 ng/L Exposed <1.7 Unexposed <1.7 ng/L Exposed 1.9 Unexposed <1.7 ng/L Exposed 1.9 Unexposed 1.9 ng/L Exposed 9.5 Unexposed 7.2 ng/L Exposed 1.6 Unexposed 1.3 ng/L Exposed 0.8 Unexposed <0.5 ng/L Exposed <1.1 Unexposed <1.1 ng/L Exposed <1.5 Unexposed <1.5 ng/L Exposed <0.6 Unexposed <0.6 ng/L	(Ranzi et al., 2013)

Country/ Study	Population	PAH/OH-PAH	Level	Reference
Poland	Pregnant women	1-OH-PYR	1-OH-PYR 0.43 µg/g creatinine	(Polanska et al., 2014)
Poland	General Population [d]	PYR BaP	<1.9 ng/L <0.5 ng/L	(Campo et al., 2014)
Portugal	Children – Boys [e]	1-OH-PYR	0.0363 µg/g creatinine	
Portugal	Children – Girls [e]	1-OH-PYR	0.0224 µg/g creatinine	(Oliveira et al., 2017)
Spain	General Population - non-smokers	1-OH-PYR	0.079 µg/g creatinine	(Bartolomé et al., 2015)
Spain	General Population - smokers	1-OH-PYR	0.184 µg/g creatinine	
Spain	General Population - non-smokers	OH-PHE	0.089 µg/g creatinine	
Spain	General Population - smokers	OH-PHE	0.317 µg/g creatinine	
United Kingdom	General Population [f]	1-OH-PYR	0.14 ng/mL No ETS subjects// 0.13 ng/ml ETS subjects	(Aquilina et al., 2010)
Czech Republic	Mothers and newborns	1-OH-PYR 3-OH-BaP	150 ng/L < 90 ng/L	(Urbancova et al., 2017)

a - Associated with Alkaline comet assay and 8-OHdG.
b - Before exposure (T1), immediately after exposure (T2), 4h (T3) and 15h (T4) after the end of the exposure to ambient air at two outdoor locations close to metallurgical industries.
c - Exposure to particulate matter from solid waste incinerator.
d - Also values for Coke Oven Workers.
e - PS1 – School 1; PS2 – School 2; There is also values for morning and night urine.
f - ETS - Environmental tobacco smoke; indoor microenvironment (home and office).

6.1.2 Occupational exposure

Since 2008, 28 studies were published reporting occupational exposure occurring in sites based in Europe using biomonitoring tools (Table 5). Most of the studies were focused on occupational settings where exposure is known to be high, such as bitumen workers and road-side construction and coke plant, aluminum electrode production plant and coal tar use.

Table 5: List of PAH/OH-PAH levels measured in different studies from EU countries in different occupational settings

Occupational settings	Exposure Scenario	Biomarker used	Median (Range of values)	References
Bitumen workers and road-side construction	Pavers	1-OH-PYR, creatinine adjusted/µg/l Median (min-max)	1.40 (0.15–7.70)	(Fostinelli et al., 2018)
	Ground workers		1.03 (0.13–6.11)	
	Roll-drivers		0.63 (0.04–3.38)	
	Truck-drivers/Others		0.26 (0.01–1.79)	
		1-OH-PYR, creatinine adjusted/ µg/g	Smokers 1.12 (0.82; 1.67) Non-smokers 0.46 (0.35; 1.51)	(Lotz et al., 2016)
		1-OH-PYR, creatinine adjusted/ µg/l	95perc - 150.4	(Marczynski et al., 2011)
		1-OH-PYR, creatinine adjusted/ng/l	1.49 (1.08–2.08) non-smokers 1.53 (1.15–2.15)smokers	(Pesch et al., 2011)
		1-OH-PYR, adjusted to creatinine (ng/g)	599 - 981	(Raulf-Heimsoth et al., 2011)
		1-OH-PYR, creatinine adjusted/ng/l	Max 430	(Rihs et al., 2011)
	Bitumen only	1-OH-PYR, adjusted to creatinine/ng/g	455 (216–827)	(Raulf-Heimsoth et al., 2008)
	Biitumen and tar		6605 (4628–11598)	
Pre-baked electrode production plant		1-OH-PYR, adjusted to creatinine/µmol/mol	0.1-2.08	(Barbeau et al., 2015)
Waste incinerator		1-OH-PYR, adjusted to creatinine/ µg/g	0.2 (<0.01 – 0.2)	(Mari et al., 2009)
Coke Plant,		1-OH-PYR, creatinine adjusted	Average of minimum and	(Barbeau et al.,

Occupational settings	Exposure Scenario	Biomarker used	Median (Range of values)	References
aluminum electrode production plant, coal tar use		nmol/mol	maximum concentrations 108 - 727	2017)
		1-OH-PYR, creatinine adjusted. µg/g	0.31 (<LOD - 7.61)	(Zajac et al., 2016)
		1-OH-PYR, creatinine adjusted/ µmol/mol	4.25 (P95) 2.15 (AM)	(Klöslová et al., 2016)
		1-OH-PYR, creatinine adjusted/ µmol/mol	Highest excretion level - 0.35 Highest post-exposure level without gloves 0.06–0.98	(Scheepers et al., 2009)
		1-OH-PYR, creatinine adjusted/ µg/g	Median 13.5	(Marczynski et al., 2009)
	Coke production		17.8 (11.9–26.6)	
	Refractory materials		39.8 (28.7–55.1)	
	Carbon electrodes		51.8 (33.5–80.1)	
	Converter workers		26.6 (14.7–48.0)	
		1-OH-PYR, creatinine adjusted/ µg/g	0.09 (0.08 – 0.49)	(Seidel et al., 2008)
	Converter infeed	1-OH-PYR, creatinine adjusted/ µg/g	9.40/P95 35.14	(Bruning et al., 2007)
	Refractories		8.29/P95 45.42	
	Coking plant		4.30/P95 24.29	
	Graphite electrodes		2.05/P95 17.50	
Metallurgy		1-OH-PYR, creatinine adjusted/µmol/mol	2.47 (0.24–11.06)	(Barbeau et al., 2014)
Firefighters		1-OH-PYR, creatinine adjusted/µmol/mol	Maximum – 0.340	(Oliveira et al., 2017)
City bus drivers and other		1-OH-PYR, creatinine adjusted/ ng/g	Average: 162.7 (SD: 187.8)	(Sancini et al., 2014)

Occupational settings	Exposure Scenario	Biomarker used	Median (Range of values)	References
outdoor workers				

There appears to be a trend for the use of the urinary metabolite 1-OH-PYR, probably because it still represents the best biomarker of occupational exposure to PAHs (SCOEL 2016). However, it should be pointed out that for evaluation of low-level occupational exposure to PAHs, it is crucial to consider intra- and inter-individual background variation in the evaluation of 1-OH-PYR. This explains why most of the studies used a control group or used two sampling moments (pre and post-shift, and sometimes during several days of the working week).

The importance of using biomonitoring tools in occupational exposures to PAHs is because these substances are known to be absorbed through skin and the levels of urinary 1-OH-PYR are particularly high in occupationally exposed populations where dermal exposure is likely (van Rooij et al., 1993; Unwin et al., 2006; RAC, 2018). Therefore, and to allow for a correct interpretation of biomonitoring data, a detailed description is needed of the tasks carried out, the duration of tasks, and the personal protective equipment worn (RAC, 2018).

Additionally, it must be consider that 1-OH-PYR is a pyrene metabolite and is therefore an indirect marker of exposure to PAHs mixtures that include BaP. The determination of BaP-specific metabolites, in particular 3-OH-BaP, can provide a more representative indication of carcinogenic risk. Recently, several improvements in the analytical method regarding sensitivity will allow using more frequently 3-OH-BaP as an exposure biomarker when exposure to BaP is expected (RAC, 2018).

The importance of choosing the correct biomarker or, most likely, the biomarkers that can really describe the PAHs mixture present in each occupational setting is of most importance since health effects depend not only of the PAH levels but also on the mixture composition.

6.2 Risk assessment for the sample populations

6.2.1 General population

As described in the methods section, we selected 9 articles with HBM data from general population, among which 3 also presented air monitoring data (Table 6). Along with that data, also the ELCR values calculated based on external exposure (air monitoring) are presented in Table 6. As referred before, the low levels of PAHs metabolites described in urine in these studies did not allow the back-calculation of exposure levels using HBM in the RAC 2018 approach, thus no values of HBM-based ELCR concerning general population are presented in this section.

Table 6: ELCR calculated based on the air monitoring levels of BaP from several EU countries , according to (RAC-Committee for Risk Assessment, 2018b).

References	Setup	Max. value BaP (µg/m^3)	Cumulative exp. (70 years)	Relative Risk lung cancer (RRx)	Max. Value ELCR
Leroyer et 2010	General Population	1.20×10-3	0.0840	1.00	3.56×10^{-5}
Oliveira et al. 2017	Children PS1 indoor	5.30×10-5	0.0028	1.00	1.57×10^{-6}
	Children PS1 outdoor	1.00×10^{-4}	0.0037	1.00	2.97×10^{-6}
	Children PS2 indoor	9.78×10^{-5}	0.0070	1.00	2.90×10^{-6}
	Children PS2 outdoor	2.12×10-6	0.0068	1.00	6.30×10^{-8}
Aquilina et al. 2010	General Population No environmental tobacco smoke	8.00×10^{-5}	0.0001	1.00	2.38×10^{-6}
	General Population environmental tobacco smoke	1.30×10^{-4}	0.0056	1.0	3.86×10^{-6}

When considering environmental air determinations, the ELCR is not high and the associated risk is low.

The median dietary exposure across European countries for average and high level dietary consumers amounted between 19.5 ng/kg b.w./day and 34.5 ng/kg b.w./day for PAH4, respectively (EFSA, 2008). For PAH8 the median dietary exposure for average and high level dietary consumers was between 28.8 ng/kg b.w./day and 51.3 ng/kg b.w./day, respectively. The calculated ELCR levels are shown in Table 7.

Table 7: Mean dietary exposure to PAH4 and PAH8 determined by EFSA (EFSA, 2008) and calculated ELCR values, based on dose–response determined by the RAC-Committee for Risk Assessment (2018)

Reference	Population	Median of daily intake (µg/kg b.w. day)	ELCR*	ELCR**
EFSA, 2008	general / mean consumers	0.0195	4.02×10^{-5}	2.79×10^{-5}
EFSA, 2008	general / high consumers	0.0345	7.11×10^{-5}	4.93×10^{-5}

*based on PAH4 dose-response: $2{,}06 \times 10^{-3}$

**based on PAH8 dose response: $1{,}43 \times 10^{-3}$

With regard to calculated ELCR based on EFSA median dietary intake and RAC cancer dose response it could be noted that cancer risk is for general mean and high consumers is around 10^{-5}. Even though there is no EU legislation setting tolerable cancer risk levels, cancer risk levels of 10^{-6} are usually seen as indicative tolerable risk level for the general population (http://ec.europa.eu/growth/content/workshop-acceptable-level-risk-workers-and-consumers-exposed-carcinogenic-substances-0_en).

Table 8: Daily intake of pyrene determined in HBM4EU, under WP12.5 (Sarigiannis & Karakitsios, 2018).

Reference	Country	Population	Median of daily intake* (µg/kg_bw/d)	Daily intake attributed to food (µg/kg_bw/d)	PAH4 dose-response for ELCR (µg/kg_bw/d)	ELCR
WP12.5, 2018	EU (Belgium)	general/ non-smokers	0.025	0.0225	2.06×10^{-3}	4.64×10^{-5}
WP12.5, 2018	EU (Netherlands)	general / smokers	0.240	0.216	2.06×10^{-3}	4.45×10^{-4}
WP12.5, 2018	EU average	general	0.050	0.045	2.06×10^{-3}	9.27×10^{-5}

*based on pyrene

The work developed in HBM4EU, under WP12.5, also showed that dietary exposure dominates exposure to PAHs, contributing to almost 90% of daily intake, except in the case of smokers (Sarigiannis & Karakitsios, 2018)..

According to Table 8, the median intake estimates for pyrene ranges between 0.025 for non-smokers in Belgium to 0.240 µg/kg bw/d for smokers in Netherlands. Intake estimates of pyrene resulted in a cancer risk around 10^{-5} for general EU population. However, the lack of B[a]P specific biomarker data was a problem for estimating intake levels of the compound that is driving PAHs mixture carcinogenicity.

6.2.2 Risk assessment for occupational exposure

Considering the data available in the 28 papers analysed, the excess lifetime lung cancer risk (ELCR) was estimated for workers based on urinary 1-OH-PYR concentrations (ECHA, 2018). The ELCR estimation (https://echa.europa.eu/fi/applying-for-authorisation/evaluating-applications) performed was target for having results distributed per occupational setting. Therefore, the data available in the papers were distributed by occupational setting groups based on the similarities of the process or conditions. The groups defined were the following: bitumen workers and road-side construction; pre-baked electrode production; waste incineration; coke and aluminium electrode production plants and coal tar use; metallurgy; firefighting; and city bus drivers and other outdoor workers.

With these estimations is possible to identify the group with higher ELCR and eventually where additional regulatory actions or investments in RMMs should be prioritize. However, they do not allow to define the type of RMMs that should be implemented since information about the most relevant exposure route is lacking.

For ELCR estimation a rationale was followed, namely:

- Considered only the HBM data (1-OH-PYR, adjusted by creatinine concentrations).

- Considered the 1- OHP median and higher values obtained to make the ELCR calculation. Normally, the median and higher values (max or P95) considered were obtained in post-shift samples in the end of the week.

- Assume that workers do not use respiratory protection equipment and there is no significant dermal exposure. This increases uncertainty in the ELCR estimations since we know that for some workplaces dermal route can have a very relevant role in total exposure to PAHs.

Moreover, it is important to consider that the predominant route of exposure will depend on the occupational setting and common workplace conditions and, although cancers occur from systemic exposure, the route of exposure influences significantly the site where local cancers occur (i.e. lung cancers can be expected to occur mainly from exposure via inhalation and skin cancers from dermal exposure) (Kroese et al.. 2001; ECHA, 2018). Considering the above, and for the papers that have air monitoring data available (BaP), the ELCR was also calculated using these data.

The following table presents the data available and the ELCR estimation only for the papers that have both kind of data, namely air monitoring and biomonitoring. As explained in RAC document (ECHA, 2018), this is possible using the survey published by Unwin et al. (2006) which involved an occupational hygiene study of 25 sites using both airborne monitoring of 17 individual PAHs and biological monitoring set the relationship between airborne BaP and urinary 1-OH-PYR. Applying the observed relationship between urinary 1-OH-PYR and airborne BaP, a level of 1-OH-PYR of 4 µmol/mol creatinine is roughly equivalent to an airborne BaP level of 0.26 µg/m^3.

Table 9: Data available on exposure to PAHs in occupational settings and calculated ELCR

Occupational settings	Exposure scenarios	Biomarker used	Median/ Max value µmol/mol creatinine;	ELCR Median/max value	References
Bitumen workers and road-side construction	Pavers	1-OH-PYR	1.24/ 6.81	$5.55x10^{-5}/2.87x10^{-3}$	Fostinelli et al., 2018
Bitumen workers and road-side construction	**Air monitoring data BaP**		**GM=$6.6x10^{-3}$**	**$3.73X10^{-5}$**	
	Biitumen and tar		6.66/11.69	$2.79x10^{-3}/5.33x10^{-3}$	Raulf-Heimsoth et al., 2008
		Air monitoring data BaP	**200**	**$1.12x10^{-6}$**	
Pre-baked electrode production plant		1-OH-PYR	0.1-2.08	Max $4.79x10^{-4}$	Barbeau et al. 2015
		Air monitoring data BaP	**Max =0. 425**	**$2.38x10^{-3}$**	
Coke Plant, aluminum electrode production plant, coal tar use		1-OH-PYR	Max =7.67	Max $3.30x10^{-3}$	Zajac et al., 2016
		Air monitoring data BaP	**Higher average =0.00079**	**$4.42x10^{-6}$**	
		1-OH-PYR	4.25 (P95) 2.15 (AM)	P95: $1.57x10^{-3}$ AM:$5.15x10^{-4}$	Klöslová et al. 2016
		Air monitoring data BaP	**Max 3.18 µg/m^3**	**$1.78x10^{-2}$**	
		1-OH-PYR	Median 13.61	$6.30x10^{-3}$	Marczynski et al., 2009
	Coke production		17.94/26.81	$8.48x10^{-3}/1.30x10^{-2}$	
	Refractory materials		40.12/55.54	$1.97x10^{-2}/2.74x10^{-2}$	
	Carbon electrodes		52.21/80.74	$2.58x10^{-2}/4.02x10^{-2}$	
	Converter		26.81/48.38	$1.30x10^{-}$	

Occupational settings	Exposure scenarios	Biomarker used	Median/ Max value µmol/mol creatinine;	ELCR Median/max value	References
	workers			^{2}/2.38x10^{-2}	
		Air monitoring data BaP	**Max 24.1 µg/m^3**	**1.35x10^{-1}**	
	Converter infeed	1-OH-PYR	4.82/P95 35.42	1.86x10^{-3}/1.73x10^{-2}	Förster et al., 2008
	Refractories		8.36/P95 45.78	3.65x10^{-3}/2.25x10^{-2}	
	Coking plant		4.33/P95 24.48	1.60x10^{-3}/1.18x10^{-2}	
	Graphite electrodes		2.07/P95 17.64	4.74x10^{-4}/8.33x10^{-3}	
		Air monitoring data BaP	**Max 44.3 µg/m^3**	**2.48x10^{-1}**	

Overall, the data indicates that occupational exposure is still higher than general population, resulting in higher ELCR values (several values higher than 1x10^{-4}). Moreover, in some studies, when using HBM data for the ELCR estimation, higher risk values are obtained (in 3 out of 7 studies) as compared with ELCR values based uniquely in air monitoring. This might indicate that for those specific workplaces dermal intake can have an important role in the total exposure to PAHs. However, these results should be considered with caution, since ELCR estimation based on air monitoring data is based in BaP concentrations and HBM data is based on a biomarker that reflects exposure to PAHs mixtures that include BaP. Considering all the uncertainties described, the results obtained donot allow stating that HBM is a more accurate tool for exposure and risk assessment and claims attention for the need to develop and apply a more specific biomarker for BaP exposure.

7 Discussion and conclusions

In the presented ELCR estimates, based on the EFSA or WP12 input data, the calculations yielded risk levels of the same order of magnitude (10^{-5}) with an exception for smokers (10^{-4}). As the risk level around 10 times lower (10^{-6}) was estimated using air monitoring data (external measurements), this indicates that using solely air monitoring data may underestimate the risk. Firstly, this might be due to the fact that dietary exposure is the main contribution to the burden of PAH metabolites and secondly this may indicate that using external exposure may be not relevant in risk calculations and that HBM data are crucial for risk assessment.

Overall, it can be concluded that general use of 1-OH-PYR as a biomarker for PYR exposure and a surrogate of other PAHs exposure, including BaP, generates some uncertainties in risk calculation.

The available approaches for assessing real-life exposure to PAHs, and the consequent risks to human population, are not satisfactory and further studies are necessary regarding specific biomarkers for BaP exposure, or methodologies more sensitive to detect small concentrations of the biomarkers already in use, should be developed to guarantee a more accurate exposure and risk assessment. In addition, considering the review of the literature, it becomes clear that, for attaining a geographical coverage, further HBM data is necessary, from more countries/regions in Europe.

Concerning occupational settings, this work claims attention for two main aspects, namely: i) the exposure levels are still high in some occupational settings and ii) there is a need for developing new occupational studies, applying a set of exposure biomarkers or a more specific biomarker for BaP exposure, which would allow a better ELCR estimation for exposed workers.

Overall, it can be concluded that the available approaches for assessing real-life exposure to PAHs, and the consequent risks to human population, are not satisfactory and further studies are necessary.

7.1 Recommendations for the regulatory risk assessment

A major advantage of HBM data is that it provides an integrated overview of the body burden selected chemicals and serve as a good approximation of aggregate exposure. However, environmental/HBM studies in EU should be standardized allowing a much accurate comparison of the PAHs levels across all Europe and also a homogeneous publication of the data. This in turn requires the use of individual HBM data, accompanied by ancillary information that would shed light on the mechanistic link between exposure dynamics and observed HBM data. This will allow a much more accurate comparison and so derived reference values based regulations, for example for PAHs in food in EU regulation to minimize exposure, the RASFF to alert consumers on potential food PAHs overload, monitoring foods production and market, improved official control of the levels of PAHs in foodstuffs, change of bad dietary practices, etc. In fact, at the moment, many studies in Europe show different approaches that in many cases could not be directly comparable.

7.2 Future prospects

The fact that the PAHs pattern largely varies in the environment and consequently in organisms might mean that for obtaining accurate data for biomonitoring purposes more than one biomarker is needed. In the same way and regarding occupational exposures probably the use of only one biomarker will not be enough since the PAHs mixture will vary between settings and biomarker selection should take this in consideration.

The most often studied biomarker for PAHs of environmental sources is 1-OH-PYR, the main metabolite of pyrene formed in mammals. Its concentration in urine is relatively high and thus easy to measure. However, PYR is not carcinogenic and monitoring of this metabolite for the purpose of risk assessment is only based on the assumption that its level to some extent correlate with BaP and other carcinogenic species co-occurring commonly with PYR in PAHs mixtures. In addition, exposure to PAHs mixture may modify metabolism or induce different effects, therefore a single metabolite may not adequately characterize exposure to PAHs.

The need of knowing the PAH levels but also on the composition of the mixture to better estimate the health effects have led to efforts to identify other biomarkers that can better represent occupational exposure. Recently, in a study developed by Barbeau and colleagues the urinary

trans-anti-7.8.9.10-tetrahydroxy-7.8.9.10-tetrahydrobenzo(a)pyrene showed to be a more specific biomarker of PAH health risks than the other metabolites previously used to perform biological exposure monitoring (Barbeau et al., 2017). This metabolite is produced by hydrolysis of BPDE, the ultimate carcinogenic metabolite of BaP which is the only PAH classified as a known human carcinogen (Barbeau et al., 2017; IARC - International Agency for Research on Cancer, 2010b).

The general use of 1-OH-PYR as a biomarker for PYR exposure and a surrogate of other PAHs exposure, including BaP, generates some uncertainties in risk calculation. For example, it was shown by Leroyer et al. (2010) that urine concentrations of 1-OH-PYR were not correlated with atmospheric concentrations of PAHs, in the environmental exposure scenarios study relevant to the general population. Conversely, some papers, e.g. Klöslová et al. (2016) and Unwin et al. (2006) confirmed good correlation between 1-OH-PYR in urine and BaP or total PAHs in air.

Additionally, new studies applying specific biomarkers for BaP exposure or a set of biomarkers for several PAHs, need to be conducted to guarantee a more accurate exposure and risk assessment.

Indeed, Urbancova et al. (2017) reported that for more accurate monitoring and risk assessment the metabolites of BaP might be considered as suitable exposure biomarkers for human studies. However, the detection of the most often targeted BaP metabolites 3-OH-BaP and benzo[a]pyrene-7-ol in urine were reported to be rather difficult, because they occur in urine at ultra-trace levels as due they are relatively non-polar and therefore are mainly excreted in *faeces*. It was also shown recently, that with regard to the inhalation exposure of the general population, and in addition to the hydroxylated metabolite of PYR or BaP, biomonitoring of PAHs should include metabolites of naphthalene, fluorene and phenanthrene (Sochacka-Tatara et al., 2018; Urbancova et al., 2017). For this purpose, methodologies more sensitive to detect small concentrations of the biomarkers need to be further developed and implemented in HBM studies.

Alongside with the analysis of urinary metabolites which are traditional biomarkers of PAHs exposure, the possibility of detecting PAHs metabolites in other human tissue like hair, for example, should also be considered (Grova et al., 2018).

In addition, considering the review of the literature, it becomes clear that, for attaining a geographical coverage, further HBM data is necessary, from more countries/regions in Europe and therefore. Additionally, new studies applying specific biomarkers for BaP exposure, or methodologies more sensitive to detect small concentrations of the biomarkers already in use, should be developed to guarantee a more accurate exposure and risk assessment. Therefore, new studies could be planned under HBM4EU.

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Annex F: PFAS risk assessment

D5.5 Substance-group specific risk assessment for PFAS

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1 Introduction

The aim of Task 5.3 in HBM4EU is to demonstrate how human biomonitoring data can be used to improve risk assessment in a regulatory context. The PFAS WP 5.3 team faced the particular case to rely on a recent scientific opinion of the Contaminants (CONTAM) Panel of the European Food Safety Authority (EFSA) using human biomonitoring (HBM) data for the exposure and risk assessment of perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) (EFSA 2018). Therefore, the approach of the WP.5.3 PFAS (per- and polyfluoroalkyl substances) team was to build on this recent and other available assessments and explore further possibilities to assess the risk to PFAS mixtures, as they are most commonly present in the human body. There are several options for approaching this task, also depending on the data origin, whether it is human, in vivo, in vitro or in silico data. These approaches and options will be described in more depth in the following sub-chapters.

The HBM4EU Scoping Document on PFAS provides background information related to the substance group and addresses the relevant policy questions (HBM4EU, 2017). Main concerns related to the substance group are that for several PFAS their “super” persistency, widespread environmental and human contamination, high mobility, long half live in humans and toxicity is proven, whereas for a variety of other PFAS in use, data on toxicity and exposure are lacking. Nowadays concerns are growing that these understudied PFAS or not yet identified compounds and/or their degradation products may reveal similar properties and/or similar mode of actions. For example, results of a recent publication indicate that some fluorinated alternatives, which are used frequently replacing the legacy PFAS, have similar or higher toxic potency than their predecessors when correcting for differences in toxicokinetics (Gomis et al. 2018).

In a toxicity ranking of various PFAS including PFOS and PFOA using dose–response curves of liver enlargement from sub-chronic oral toxicity studies in male rats, the relative toxicities varied considerably depending on whether external or internal dose descriptors were used. Potency differences among the studied PFAS including fluorinated alternatives in some cases disappeared when internal doses were used for the assessment indicating the important role of toxicokinetics (Gomis et al. 2018).

Human exposure assessment of a specific chemical is often based on data derived separately for various relevant sources. For instance, exposure via food is often derived from data on chemical concentrations in various food items and average data for human food intake patterns, whereas exposure to the same chemical via cosmetics is assessed separately. Assessing aggregate exposures is thus a challenge and large data gaps still exist on human exposure to chemicals. Human internal exposure assessed via HBM is a measure of aggregated exposure but are not routinely used in chemical risk assessment. Yet HBM data is increasingly being gathered all over Europe (HBM4EU) and US (NHANES), presenting a great opportunity to evaluate human exposure integrated across sources. In this report we have used aggregated HBM data for PFAS to be used in mixture risk assessment and for informing risk assessment together with in vitro data.

The aims of this report were:

- To provide a summary of existing risk assessments of PFAS
- To perform a preliminary mixture risk assessment of PFAS using HBM data combined with experimental toxicity data on relative potency
- To investigate whether PFAS can be grouped by alternative ways for mixture risk assessment
- To investigate whether HBM data together with in vitro data can inform risk assessment for PFOS

2 Existing Health Based Guidance Values (HBGV)

PFAS have unique chemical properties, PFASs bind to proteins and partition to phospholipids. The elimination kinetics are highly species dependent, with humans showing the longest half-lives (several years) compared to animals. For example, the half live of PFOS in laboratory rodents is 25 days compared to monkeys with 45 days and humans 1500 days (Li et al. 2018). A recent publication reports an estimated elimination range of 10.1 to 56.4 years – median 15.3 years for chlorinated polyfluoroalkyl ether sulfonic acids (Shi et al., 2016). The CLP human health hazard classifications of perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) – are carcinogenic (Carc. 2, suspected human carcinogens, such as kidney and testicular), toxic for reproduction (Repr. 1B, presumed human reproductive toxicants; Lact., may cause harm to breast-fed children), toxic to specific target organs (STOT RE 1, specific target organ toxicity – repeated exposure) and acute toxic (Acute Tox. 3-4) for different exposure routes. Primary target organ for these substances is the liver. The potential of these substances to trigger the PPARα receptor (e.g. leading to lower serum cholesterol levels in laboratory animals) and other nuclear receptors (PPARδ, ERα) lead to a variety of effects in laboratory animals and also humans. In 2017, RIVM published a review of epidemiological data “PFOA exposure and health” and identified a relationship between exposure to PFOA and higher total cholesterol concentrations in blood, higher concentrations of the liver enzyme ALT in blood and a lower birth weight as of clearest evidence for associations of health outcomes with PFOA exposure. Also other associations with less clear evidence were described: association with higher blood concentrations of other liver enzymes, LDL-cholesterol and uric acid, a higher risk of chronic inflammation of the bowel (ulcerative colitis), testis and kidney cancer, as well as pregnancy-induced hypertension and preeclampsia. Furthermore, associations have been found between exposure to PFOA and a decreased vaccination response, changes in concentrations of thyroid hormones in blood and thyroid disease (RIVM, 2017). In 2018 the **European Food Safety Authority (EFSA)** published a scientific opinion on the risk to

human health related to the presence of PFOS and PFOA in food. The recent EFSA assessment was originally intended to cover a group of 27 PFAS, but only for 11 PFAS were sufficient human exposure data available. The first part of the assessment focuses on PFOA and PFOS, another assessment of further PFAS compounds will follow by the end of 2019[17]. It is foreseen since these substances are often present as mixtures in the food chain, EFSA's development of frameworks for assessing combined exposure to multiple chemicals will feed into this work. The derived health based guidance values (HBGV) were based on human epidemiological studies. The EFSA CONTAM Panel has evaluated HBM data across Europe as well as time trends. HBM data were used for exposure assessment and provided the basis for risk assessment. Physiologically based pharmacokinetic (PBPK) modelling was performed in order to estimate the relationships between serum concentrations of PFOA and PFOS and dietary intakes. The potential critical human endpoints identified were:

- increased serum cholesterol (indicating an increased risk of future cardiovascular disease)
- increased prevalence of abnormal serum levels of alanine aminotransferase (indicating an effect on hepatocytes).
- decreased antibody response after vaccination (indicating impaired immune function).
- decreased birth weight (which may increase risk of low birth weight (below 2500 g) and risk of future disease).

For PFOS, the increase in serum total cholesterol in adults, and the decrease in antibody response at vaccination in children were identified as the critical effects. The respective Benchmark Doses for a 5% increase (BMDL5) in cholesterol were in terms of serum concentration was 21–25 ng/mL, corresponding to an estimated chronic daily intake of 1.7–2.0 (median 1.8) ng/kg bw/day. For children the lowest BMDL5 was established for delayed antibody response after vaccination (10.5 ng/mL). The CONTAM Panel established a provisional tolerable weekly intake (pTWI) for PFOS of 13 ng/kg bw/week.

For PFOA, the CONTAM Panel considered the increase of serum cholesterol to be the critical effect. The respective BMDL5 values were 9.2–9.4 ng PFOA/mL plasma; corresponding to an estimated chronic intake of 0.8 ng/kg bw/day. The CONTAM Panel established a provisional tolerable weekly intake (pTWI) for PFOA of 6 ng/kg bw/week.

At the moment there are ongoing discussions concerning this opinion at European level, this is also documented in the minutes of an expert meeting on PFOS and PFOA in food assessment. The institutions involved in the expert meeting were EFSA, ECHA, BfR, Danish EPA, and RIVM as well the experts of the CONTAM panel.

Main points of these discussions are:

- The suitability of the information in the epidemiological studies available for deriving a Point of Departure (PoD);
- The assumptions made in the derivation of the PoD;
- The inconsistency of the applied BMD analysis and the existing EFSA guidance.
- The safety of a long breastfeeding period for young children regarding possible effects on reduced antibody formation after vaccination.

[17] https://www.efsa.europa.eu/sites/default/files/news/efsa-contam-3503.pdf

- The possible overestimation of estimated intake of PFOS/PFOA if a substantial proportion of samples originated from "hotspot regions".

The experts concluded that further clarifications will be needed and shall be provided in the scientific opinion, as well as a reference to the meeting minutes. Further discussions and future collaborations were strived for[18].

The **Agency of toxic substances and disease registry (ATSDR, US)** has recently published a Draft Toxicological Profile of PFAS (ATSDR, 2018). Provisional minimal risk levels (MRL) were derived only for intermediate oral exposure to PFOS, PFOA, PFHxS and PFNA. MRLs are usually derived by using the No Observable Adverse Effect Level (NOAEL)/uncertainty factor approach and are intended only to serve as a screening tool. The approach for deriving the MRL was to first identify the most sensitive endpoints in human epidemiology studies. Further, laboratory animal studies that had evaluated dose-response relationships for the identified toxicity targets were used in order to estimate a PoD and their corresponding serum perfluoroalkyl levels. Human equivalent doses (HEDs) were then calculated using the assumption that a serum concentration resulting in an effect in a laboratory animal would also result in an effect in humans. An empirical pharmacokinetic model was used to estimate a human dose associated with this serum concentration for PFOA and PFOS. Serum concentrations in laboratory animal studies were used to calculate the HEDs for PFHxS and PFNA. Uncertainty factors informed by comparison of the PoD to serum perfluoroalkyl levels reported in epidemiology studies were applied. PFAS MRLs were calculated only for intermediate (15–364 days) durations and for the oral exposure route. The derived MRLs were 3 ng/kg bw/day for PFOA and PFNA, 2 ng/kg bw/day for PFOS, and 20 ng/kg bw/day for PFHxS. For PFOS a NOAEL based on delayed eye opening and decreased F2 rat pup body weight (NOAEL predicted TWA serum concentration of 7.43 µg/ml) was selected as critical effect. For PFOA the selected critical effect was altered activity and skeletal alterations in offspring in mice at a predicted TWA serum concentration of 8.29 µg/ml. The immune system has been identified as a sensitive target organ in intermediate-duration animal studies. Immunotoxicity data for PFOA with respect to reduced IgM antibody response in mice were also available and eligible for benchmark modelling resulting in a Benchmark dose level $BMDL_{1SD}$[19] of 12.23 µg/ml (De Witt et al. 2016) or 33.49 µg/ml (De Witt et al. 2008) and an applied uncertainty factor (UF) of 30. For PFOS measured serum levels associated with altered immune responses are approximately 1–10 times lower than the serum concentration predicted to occur at the NOAEL for developmental effects (candidate POD of 1.2 µg/ml according to ATSDR, 2018) justifying an extra modifying factor of 10 to account for susceptibility of these effects. ATSDR did not derive chronic MRLs for PFOA and PFOS, as no chronic studies investigating immune toxicity in laboratory animals were available. The intermediate oral MRL of PFHxS was based on a NOAEL for thyroid follicular cell damage with an estimated TWA (time-weighted average) serum concentration of 73.22 µg/ml selected as a PoD. The intermediate oral MRL for PFNA was based on decreased body weight in pups and developmental delays at a predicted NOAEL TWA serum concentration of 6.8 µg/ml served as a POD. The uncertainties factors (UF) applied were 3 for interspecies differences including dosimetric adjustments, 10 for intraspecies differences

[18]https://www.efsa.europa.eu/sites/default/files/news/efsa-contam-3503.pdf

[19] BMDL = 95% lower confidence limit on the BMD (subscripts denote benchmark response: i.e., $_1$ = exposure concentration associated with 1% extra risk); SD = standard deviation;

and a modifying factor of 10 for database limitations (PFHxS, PFNA) or 10 for the concern that immunotoxicity may be a more sensitive endpoint than developmental toxicity (PFOS) or 10 for use of a LOAEL (PFOA). ATSDR did also not derive chronic MRLs for PFHxS and PFNA as no chronic oral toxicity studies in rats were available (ATSDR, 2018).

Human reference values generated in Borg et al. (2013) refer to internal dose measurements at the NOAEL for liver effects for PFHxS, PFOS and PFOA and the LOAEL for PFNA (this was accounted with an addition uncertainty factor of 3) in animal studies: The NOAEL for PFOA (0.06 mg/kg bw or 7.1 µg/ml serum) is based on liver hypertrophy and for PFHxS (1 mg/kg bw or 89 µg/ml serum) on liver hypertrophy and relative liver weight increase of 16%, but cholesterol in rats was reduced at lower doses for PFHxS already (Butenhoff et al. 2009). PFNA induced relative liver weights >50% at the LOAEL of 0.83 mg/kg bw or 28.5 µg/ml serum in mice compared to control (Wolf et al. 2010). For PFOS hepatocellular hypertrophy and vacuolation at week 14 were observed at 0.024 mg/kg bw or 4.04 µg/ml serum in male rats (Butenhoff et al. 2012).

The uncertainty factors applied were 2.5 for interspecies, 10 for intraspecies differences (or 5 for occupational exposed individuals) and a factor of 2 for extrapolation from subacute and subchronic to chronic exposure. For compounds with no toxicity data, a read across of equivalent molar concentrations from similar PFAS with different chain lengths were applied. A hazard index approach comparing the serum concentrations with target references doses was used to highlight exposures of concern, and summed to derive a Hazard Index. Thus, this approach represents a mixture toxicity approach within this group of compounds perceived to follow common target organ(s)/mode of action(s) concerning liver toxicity. It was developed as a first attempt of a cumulative health risk assessment of a large number of PFAS present in the Swedish population. The authors concluded there was no concern for hepatotoxicity for the general population, but concerns for immunotoxicity and effects on mammary gland development were identified for PFOS and PFOA.

The **committee for risk assessment (RAC) of the European Chemicals Agency (ECHA)** established a 'Derived No Effect Level$_{internal}$' (DNEL$_{internal}$) of 800 ng/mL serum for PFOA for the general population (ECHA, 2015). The DNEL was based on a mouse study where a decreased pup growth rate was observed. Within the Annex XV proposal for the restriction for PFOA, PFOA salts and PFOA-related substances prepared by the German and Norwegian competent authorities within REACH and CLP several DNELs were proposed. Depending on the endpoint and species the DNELs$_{internal}$ varied between 0.3 ng/ml (based on reduced birth weight in a human study) to 277 ng/ml (based on reduced neonatal survival in mice) for the general population, and 0.7-555 ng/ml for workers, respectively.

Some PFAS in current use are not very persistent by themselves but have the ability to degrade to persistent perfluorinated carboylic acid or sulphonates in humans and the environment. These precursors should be taken into account in risk assessments and therefore the so-called **arrow head approach** has been developed by **ECHA.** This approach takes into account that a diversity of PFAS precursors like fluorotelomer alcohols, fluorotelomer acrylates ultimately degrade into substances of concern (e.g. perfluorcarboxylic and perfluorosulfonic acids). This approach has been successfully applied for the restriction of PFOA (ECHA, 2018a), C9-C14 PFCAs (ECHA , 2018b) and is now applied to PFHxS and short chain PFAS, which are used as alternatives for the legacy PFAS (PFOA, PFOS). The main aim is to restrict and address precursor substances by risk management measures for the respective substances.

A similar approach has been applied for the proposal of a prohibition of PFOA and PFHxS and related substances under the **United Nations Environment Programme (UNEP) Stockholm Convention.**

The health based **HBM Guidance (HBM I) values developed by the German Human Biomonitoring Commission** are marking a level of protection at which "adverse health effects are not expected". The derivation of the HBM I values were derived in a weight of evidence approach, addressing relevant endpoints (fertility and pregnancy outcomes, weight of new-borns at birth, lipid metabolism, immunity after vaccination, immunological development, hormonal development, age at puberty/menarche, thyroid metabolism) and using POD ranges by evaluating human and animal data due to heterogeneity of the reported data. The HBM Commission set HBM I values for PFOA and PFOS in blood plasma at 2 ng PFOA/ml and 5 ng PFOS/ml, respectively, based on neurodevelopmental effects (Apel et al., 2017).

RIVM, the National Institute for Public Health and the Environment of the Netherlands established a HBGV for PFOA based on liver toxicity in rats after sub-chronic exposure. A tolerable daily intake of 12.5 ng/kg bw/day was established and subsequently translated to an internal health limit of 89 ng/ml serum. This limit value is based on liver toxicity in rats with a NOAEL of 0.06 mg/kg bw/day extrapolated to chronic human inhalation exposure using kinetic modelling that takes into account accumulation of PFOA (Zeilmaker et al., 2016).

Results of the Risk assessment for PFOS and PFOA (EFSA)

The CONTAM panel is currently assessing the risks to human health related to the presence in food of perfluoroalkylated substances other than PFOS and PFOA. The indicative timeline for this is December 2019. EFSA stated that, until such time, the conclusions and derived tolerable weekly intakes (TWI) shall be considered provisional.

The EFSA CONTAM-Panel concluded in its risk assessment:

…."*For **PFOS**, mean LB (lower-bound)[20] dietary exposure ranged from 1.3 to 20.9 ng/kg bw per week, across age groups and surveys. The high (95th percentile) LB exposure ranged from 3.5 to 165.9 ng/kg bw per week. Therefore, a considerable proportion of the population exceeds the TWI of 13 ng/kg bw per week, by up to 1.6- and 13-fold, for mean LB and high LB exposure, respectively. For PFOS, at the UB (upper-bound)[21], the TWI is exceeded in all surveys at mean exposure, and the high UB (95th percentile) exposures exceed the TWI from 1.7- to 15-fold across surveys and age groups.*

*For **PFOA**, mean LB dietary exposure estimates range from 1.5 to 18.3 ng/kg bw per week. The high (95th percentile) LB exposures range from 3.4 to 37.6 ng/kg bw per week. Therefore, a considerable proportion of the population exceeds the TWI of 6 ng/kg bw per week, by up to 3- and 6-fold for mean LB and high LB exposure, respectively. For PFOA, at the mean UB, the TWI is exceeded 1.4- to 14-fold across surveys and up to 28-fold at the high UB (95th percentile) exposure for toddlers. Therefore, it is clear that a considerable proportion of the population exceeds the established TWIs for PFOS and PFOA. The exceedances of the TWIs for PFOS and PFOA at LB exposure estimates are of concern."*

[20] Lower bound concentrations are calculated by setting all non detectable and non-quantified results equal to zero
[21] Upper-bound concentrations are calculated on the assumption that all the values below the LOQ are equal to the LOQ and all non detectable results equal to the LOD.

However, as depicted in table 1 comparing the HBM data from the individual European HBM studies across countries within 2007-2015, which were compiled in the EFSA assessment with the BMDL5 values in adults and children, it can be seen that the median PFOA and PFOS concentrations are well below the BMDL5 values. Only the maximum levels of PFOS reported in adults are exceeding the BMDL5. For PFOS, the median of the median concentrations in serum/plasma is 7.7 ng/mL (range 1.7 -27.4 ng/mL) and 3.2 ng/mL (range 0.49–8.6 ng/mL) for adults and children, respectively. Similarly, medians of the median concentrations for PFOA are 1.9 ng/mL (range 0.76–4.9 ng/mL) and 3.3 ng/mL (range 0.49–6.9 ng/mL). However, the maximum levels in individual studies lie far above: e.g. 80.8 ng/ml for PFOA and 392.3 ng/mL for PFOS in adults and 19.5 ng/mL for PFOA (95th percentile) and 23.0 ng/mL for PFOS in children (EFSA, 2018).

Levels in occupationally exposed adults are considerably higher and exceeding the BMDL5 up to about 700-fold for PFOA and 300-fold for PFOS. Also levels in blood from populations with elevated drinking water exposure are exceeding the BMDL5 values, which is especially relevant for vulnerable population groups such as pregnant women and children (EFSA, 2018).

Table 1: Serum concentrations of PFOS and PFOA in European adult and children populations based on studies reported in EFSA 2018 and comparison with the derived BMDL5 levels by EFSA.

Age group	Adults		Children	
Concentration	ng/ml			
	PFOA	PFOS	PFOA	PFOS
BMDL5	9.2-9.4	21-25	9.2-9.4	10.5
Median*	1.9	7.7	3.3	3.2
Mean*	2.1	7.5	3.3	3.3
Minimum*	0.76	1.7	0.49	0.49
Maximum*	4.9	27.4	6.9	8.6
Number of studies	32	32	8	8

*statistics based on medians reported from the individual studies (EFSA, 2018). The maximum and minimum values do not reflect individual levels (see text for these values).

3 HBM-based preliminary mixture risk assessment of PFAS

Both in the background exposed population and among those exposed through contaminated drinking water (like in Sweden/Ronneby and several other regions in Europe) people are exposed to several PFAS, which tend to be correlated to varying degree. Given the fact that several PFAS have been shown to affect the same endpoint (e.g. liver weight in rodents, cholesterol in humans, effects on reproduction and development, immunotoxicity) the use of one-compound-at-a-time approach for risk assessment is likely inadequate.

3.1 Methodology

The epidemiology database consists of health evaluations of subjects exposed in occupational settings (primarily PFOA and PFOS), highly exposed residents living near a PFOA production facility, and studies of the general population. The most commonly examined endpoints examined in the epidemiologic studies were developmental, reproductive, and immunological effects as well as effects on the liver.

The component-based approach was used to assess the cumulative risk of a mixture of PFAS. Component-based approaches are preferred methods when dose response data for specific toxicity endpoints of individual components exist, so that substances can be assigned to cumulative assessment groups (CAG). Ideally, mode of action (MOA) information is used to select CAGs using assumptions e. g. of additivity (dose addition, response addition) amongst the chemicals. Since MOA data are rarely available, the scientific criteria for setting CAGs are often based on target organ toxicity (EFSA draft 2018c, EFSA 2013).

Experiments have shown that chemicals, when exerting similar effects, typically act in a dose-additive manner, and far less frequently in synergistic or antagonistic ways (Kortenkamp et al. 2009; Boberg et al. 2019). Under that assumption, efficacies of chemicals can be added together, as long as the potency of the individual chemicals is appropriately accounted for. As dose response curves are usually sigmoidal (if a sufficient dose range is covered), the combination of many chemicals at low levels (located at the lowest horizontal part of the dose response curve) can give rise to a pronounced adverse mixture effect (corresponding to a move up to the steeper part of the dose response curve). This phenomenon has been observed in cellular systems (Silva et al. 2002) and animal studies (Howdeshell et al. 2017; Conley et al. 2018).

The assumption that chemicals act additively and behave as if they were a simple dilution of each other has resulted in the development of methods for cumulative risk assessment using various approaches: Hazard Index (HI), point of departure index (PODI), relative potency factors (RPF) and toxicity equivalency factors (TEF). These concepts have been described in detail in the “State of the Art Report on Mixture toxicity” mandated by the European Commission (Kortenkamp et al. 2009). The HI is defined as the sum of the hazard quotients (HQs) of the individual components of a mixture, in which each HQ is calculated as the ratio between exposure to a chemical and the respective reference values (EFSA draft, 2018c). If the HI > 1 then the concentration of the mixture components exceeds the level considered to be acceptable. The method offers flexibility in applying different uncertainty factors (if applicable) when defining a reference value for the individual substances from e.g. animal studies (Kortenkamp et al. 2009). When compared to the alternative model ‘independent action’, the assumption of concentration or dose addition is often considered a worst-case

scenario, if synergistic effects are not likely in regulatory frameworks (ECHA, 2017, Rotter et al. 2019).

In order to evaluate human risk to mixtures of PFAS, we decided to focus on the four PFAS that currently constitute the majority of PFAS in human blood according to our present knowledge: PFOA, PFOS, PFNA and PFHxS. We identified human exposure data for various segments of the human population and calculated HIs using HBM data.

In our first PFAS mixture risk assessment, we used human hazard data for PFOA and PFOS and estimated human hazard data for the other PFAS by use of knowledge on relative potency from rodent data. As the hazard data we used data from the recent EFSA risk assessment of PFOS and PFOA, in which the BMDL5 for cholesterol increases in the human population was identified as the critical effect. Similar BMDLs for PFNA and PFHxS are not available and therefore we used the recently developed Relative Potency Factors (RPF) by RIVM (cf. Table 2). The RPFs are based on liver toxicity observed in animal studies and fitted dose-response curves were used to calculate the BMDL10 (Zeilmaker et al. 2018). To calculate the RPFs, it was assumed that PFAS display similar toxicity, differ only in potency, and do not interact. We also rely on these assumptions.

The established RPFs are based on external dose metrics (RIVM 2018). A recent study by Gomis et al. (2018) illustrated that, when converting external doses of four PFAS (PFBA, PFHxA, HFPO-DA, and PFOA) to internal serum and liver concentrations, this resulted in reduced variability in the dose-response curves for liver enlargement. Therefore, it may be concluded that the relative internal potencies of these substances differ much less than their external relative potencies, as part of the (external) potency differences may be due to differences in bioavailability and absorption. Hence, the uncertainty in the quantitative outcome, when applying external RPFs to internal PFAS serum concentrations, is very high.

In addition, the use of the RPF approach has a high uncertainty, as the pTWI EFSA is based on cholesterol increase, not on liver weight increase, being the effect on which the RPF was based (Table 4).

Secondly, we did a mixture risk assessment, in which we used rodent data for the relative potency. Health based reference values for PFNA and PFHxS have been derived recently by ATSDR, and therefore values for PFNA, PFHxS as well as for PFOS and PFOA were used without the RPF approach. For comparison with internal HBM plasma concentration the POD for MRL setting divided by the UF as determined by ATSDR (2018) were calculated as a reference dose (RfD) in Table 2. Additional reference values derived based on liver toxicity by Borg et al. (2013) were applied for comparison. Please see Table 2 for the compilation of effect descriptors by the various agencies and authors.

In addition the HBM I values for PFOA and PFOS derived by the German HBM Commission (cf. section 1.1) were used for comparison and additional interpretation of the used HBM data.

The reference values or HBGV differ for the four PFAS depending on the methodology used for threshold derivation, the species that was used to determine the effect descriptor, selection of critical effect (and UFs) as well as availability of data. While MRL and RPFs refer to an external dose descriptor, all other reference values were calculated based on internal serum concentration. This might explain the quite different potency observed for PFHxS and PFNA with regards to the RPFs and the internal based RfD in Table 2. Associated uncertainties with these effect descriptors are further discussed in chapter 3.2.

For the derivation for PFHxS and PFOA of the RIVM RPF and RfD of Borg et al. (2013) the same animal studies were used. However derivation of RPFs include BMD modelling and refer to external dose levels, whereas Borg et al. used NOAELs and internal serum concentrations in the animal studies to calculate RfDs (HBM-GV). PODs derived by ATSDR were obtained by estimation of TWA serum concentrations that explain the slight differences in Table 2 for the POD of PFHxS despite that the same dose level for thyroid and liver effects of 1 mg/kg bw were used in both the assessments.

Table 2: Summary of effect descriptors of several PFAS by various authors and institutes

	pTWI (ng/ml serum) EFSA, 2018§	DNEL (ng/mL serum) ECHA, 2015	$RfD_{chronic}$ liver toxicity (ng/ml serum) Borg et al. 2013	POD (ng/ml serum) Borg et al. 2013	Applied UF Borg et al. 2013	$RfD_{internal, intermediate}$ (ng/ml serum) modified from ATSDR 2018	POD (ng/ml serum) ATSDR 2018	Applied UF ATSDR, 2018	$MRL_{intermediate}$ (ng/kg bw/day) ATSDR, 2018	HB-GV (ng/ml serum) RIVM 2018 Zeilmaker et al., 2016	RPF RIVM, 2018
PFHxS	-	-	1 780 (3560)*	89 000	50 (25)*	244	73 220	300	20	-	0.6
PFOS	21-25 (adults) 10.5 (children)	-	162 (323)*	4 040	50 (12.5)*	25	7 430	300	2	-	2
PFOA	9.2-9.4	800 (1600)	142 (284)*	7 100	50 (25)*	28	8 290	300	3	89	1
PFNA	-	-	190 (380)*	28 500	150 (75)*	23	6 800	300	3	-	10

*) Values for occupational workers in parenthesis; §) These values are based on human data. The remaining values are based on animal data.

3.2 Mixture risk assessment of four PFAS based on human biomonitoring data and animal hazard data

A number of pseudonymised HBM data sets from five different EU countries were compared to the EFSA HBM-GV and other endpoints/health effect descriptors as outlined in Table 3. The HBM data were pseudonymised, as for some HBM data sets, quite high HI's were found. Despite the assumptions (some of them which are disputable) and the uncertainties that go with them, prudence is necessary in identifying these countries without notifying the data set owners upfront.

For a preliminary mixture risk assessment of PFOA, PFOS, PFNA and PFHxS, we calculated the HI, and results are illustrated with one subset, the median PFAS serum level in a background exposed adult population. While it is clear that the derivation of the intermediate human reference values by ATSDR represent dissimilar targets and modes of actions, it can be used as a first screening approach in MRA. Even if the reference doses are based on different endpoints, a preliminary HI is calculated that include HQs for each compound based on various endpoints. If the HI turns out to be below 1, there is no need to group the chemicals according to their specific effects and re-calculate the HI. This approach is already applied in regulatory decision making for biocides within the EU (ECHA, 2017).

Table 3: Various endpoints/health effects used for HI approach

HBM-GV	Toxicological endpoint/Human effect	Substance	HBGV ng/kg bw/d ng/kg bw/w	Internal reference dose ng/mL serum
ECHA 2015 **DNEL Internal**	Mouse: Decreased pup growth rate	PFOA	-	800
RIVM 2016 **HBGV (ext + int)**	Rat: Liver effects	PFOA	12.5 (per day)	89
EFSA 2018 **pTWI (ext + int)**	**Human: Increase in serum cholesterol**	**PFOA** **PFOS**	**6 (per week)** ***0.86 (per day)*** **13 (per week)** ***1.86 (per day)***	**9.3** **23**
ATSDR (2018) **MRL (ext + int),**	Mouse: Altered activity and skeletal alterations in offspring	PFOA	3 (per day)	28*
	Rat: Delayed eye opening and decreased F2 rat pup body weight	PFOS	2 (per day)	25*
	Rat: Thyroid effects	PFHxS	20 (per day)	244*
	Mouse: Decreased body weight and developmental	PFNA	3 (per day)	23*

HBM-GV	Toxicological endpoint/Human effect	Substance	HBGV ng/kg bw/d ng/kg bw/w	Internal reference dose ng/mL serum
intermediate exposure*	delays in mice			
Borg et al. (2013) **RfD (int)**	Rat and mice: liver hypertrophy, relative liver weight increase >15%, vacuolation	PFOA	-	142
		PFOS	-	162
		PFHxS	-	1780
HBM I value –German HBM Commission	Mouse: neurodevelopmental effects and skeletal effects in offspring of orally treated mouse dams	PFOA	-	2
	Rat: Delayed eye opening and decreased pup body weight in F2 generation	PFOS	-	5

In the table below, a preliminary uncertainty description is provided with respect to the various assumptions underlying the HI methodology as applied.

Table 4: Qualitative attribution of uncertainty sizes linked to various underlying assumptions

No	Approach, assumption and uncertainties involved	Uncertainty
1	The HI approach is applied for **one single and identical endpoint/effect** for comparing the various PFAS. This may not necessarily be the most sensitive effect/endpoint for each individual PFAS member. **This is a basic assumption as used quite broadly in literature.** There are four options used in Table 5:	Low
	a. *With the RPF approach: Use of ECHA DNEL internal (2015); based on decreased pup weights in mice.*	*See below*
	b. With the RPF approach: Use of RIVM HBGV internal (2016); based on liver effects in rats.	*See below*
	c. With the RPF approach: Use of EFSA HBGV internal for PFOS and PFOA (2018) and using the RPF approach for PFHxS and PFNA in comparing its potency to PFOA.	*See below*
	d. Use of internal RfD for PFOA, PFOS, PFHxS and PFNA based on liver toxicity by Borg et al. (2013)	*See below*
2	The HI approach can be applied to **different effects/endpoints** for screening purposes (Tier 1) because it is conservative. The tiered approach in MRA including Tier 1 is applied in regulatory frameworks in the EU. There is one option used in Table 5:	*Low to moderate*
	e. Use of internal RfD for PFOA, PFOS, PFHxS and PFNA based on different targets/effects by ATSDR (2018)	*See below*
3	PFOA is used as the Index Reference Compound to compare the other PFAS to by using the RPF approach as developed by RIVM.	Medium
4	The RPF approach, developed for external exposure and expressed as external HBGVs can be used with internal exposure levels **(1c).** It is assumed that the toxicokinetics of all PFAS in the mixture is linear and sufficiently similar both in animals and humans. There is a large difference in elimination rates across species. As this assumption is uncertain, the quantitative outcome of the HI is uncertain.	High
5	This RPF as developed by RIVM and based on liver effects can be used using the RIVM Internal HGBV for PFOA (as based on liver effects) as well as the EFSA Internal HBMGV for PFOA being the internal level for the pTWI = preliminary TWI (assuming that increase of serum cholesterol is mechanistically linked to the general 'liver toxicity') **(1c). As this assumption needs further scrutiny for using the EFSA pTWI serum level for PFOA** (what are the more specific liver effects that the RPF approach is based on, is it justified to assume a mechanistic link to increase in serum	Moderate

No	Approach, assumption and uncertainties involved	Uncertainty
	cholesterol), **the quantitative outcome is uncertain.**	
6	Use of the **ECHA DNEL Internal (1a)** is very uncertain as based on decreased pup weights in mice, which is a completely different effect in another species than the combination used to develop the RPF approach.	Very high
7	Use of the **RIVM HBGV (1b).** This use is linked with uncertainty as only based on a general mode of action comparison, but not on a mechanism of action or Adverse Outcome Pathway assessment.	High
8	Use of the **EFSA Internal pTWI for PFOA and PFOS (1c)**. However, there are diverging opinions regarding the EFSA Opinion on PFOA and PFOS that mainly pertain to the use of dose-response modelling on the data in the Steenland et al (2009) study and the exclusion of low-exposed (less than 1 year) human samples in the Steenland et al (2009) study.	Low
9	Use of **internal RfD from Borg et al. (1d).** This use is linked with uncertainty as the MoA for rodent heptatoxicity is still not fully established. Liver effects observed in rodents differ from those reported in humans. Endpoints with lower effect levels than hepatotoxicity were identified for PFOS and PFOA. For PFNA and PFHxS no chronic studies are available and PoD for PFNA is based on a LOAEL. Values were not expressed in moles.	High
10	Use of internal RfD for PFOA, PFOS, PFHxS and PFNA based on the **lowest critical effects by ATSDR (1e).** This use is linked with uncertainty because rodent data was used. No chronic MRL estimates were derived, so reference values only apply to intermediate exposure situations. Additional UF were introduced to account for possible more sensitive effects e.g. immunotoxicity.	High
11	The resulting modified HBM internal exposure values are then divided by an HBGV Internal to provide a HQ per PFAS substance.	Low
12	Assumption of a parallel and linear dose-response curve for all individual components in the mixture along the whole exposure distribution. This assumption is uncertain as it is known that many effects exhibit a sigmoidal dose-response curve. Some substance exposure combinations maybe in the steep part of the curve, but others only at the lower, much more flat end.	Moderate
13	Assessment outcome focus on 4 PFAS from two classes and other PFAS compounds detected in human samples were not considered.	High

Risk assessment outcomes vary considerable depending on which HBGVs are used, critical endpoints, available data and the methodology used. HBM data sets comprise different population groups including newborns, background and hot spots of the general population and occupationally exposed adults. For most HBM data sets used, the HI-values were below 1, which is typically due to the use of rodent hazard data. When human hazard data was used in combination with HBM data, the HI was above 1. Especially for occupational settings, the HI's were above 1, albeit often a HQ for an individual congener was already >1 as well.

For the purpose of illustration, one result table is shown below in Table 5.

Table 5: Example of HI calculation based on a pseudonymised data set (adult blood serum)

Adult Blood Serum								
2012-2015			**PFOA**	**PFOS**	**PFNA**	**PFHxS**		
Exposure	HBM level		2,81	7,52	0,86	1,8	ug/l plasma	
	RPF (RIVM, 2018)		1	2	10	0,6		
	PFOA units		2,81	15,04	8,6	1,08		
RA using HI for mixtures		µg/l serum	**HQ**	**HQ**	**HQ**	**HQ**	**HI**	
and RPF approach	*DNEL ECHA RAC*	*800*	*0,00*	*0,02*	*0,01*	*0,00*	***0,03***	***HI Based on DNEL ECHA RAC for PFOA***
	HBLV RIVM	*89*	*0,03*	*0,17*	*0,10*	*0,01*	***0,31***	***HI Based on RIVM HBLV for PFOA***
	EFSA 2018	*PFOA: 9.3, PFOS: 23*	*0,30*	*0,33*	*0,92*	*0,12*	***1,67***	***HI Based on EFSA pTWI internal (PFOA, PFOS)***
without RPF approach	RfD Borg et al.	PFOA: 142, PFOS: 162, PFNA: 23, PFHxS: 1780	*0,02*	*0,05*	*0,00*	*0,00*	***0,07***	**HI based on Borg et al. (2013) liver toxicity**
	RfD based on ATSDR	PFOA: 28, PFOS: 25, PFNA: 23, PFHxS: 240	*0,10*	*0,27*	*0,04*	*0,01*	***0,42***	**HI based on ATSDR (2018) mixed effects**
HBM I value			1,41	1,50	nd	nd	**nd**	**HBM I value based on Apel et al. (2016)**

The resulting HI values go with moderate to large uncertainties and are provided for illustrative purposes. The estimated (qualitative size of the) uncertainties are provided in Table 4. These uncertainties might be correlated and therefore, further quantification of the uncertainties and use of quantitative uncertainties might be used to provide worst case confidence intervals of HI's.

4 Can PFAS be grouped for mixture risk assessment by alternative ways?

4.1 Background

In order to implement mixture risk assessment of chemicals, it is important to have tools available for grouping of chemicals. This is important for chemicals to which the human population is exposed.

For some chemicals, we have in vivo data to use for grouping into cumulative assessment groups (CAGs) according to their adverse outcomes, whereas for others we are lacking in vivo data, and especially for those it is important to investigate alternative tools for grouping purposes. The PFASs constitute a group of > 4000 chemicals and we are lacking risk or hazard data for most of them. If we can find reliable computational tools to predict the hazards that would be great; ideally these should be quantitative for calculation of hazard quotients. However, qualitative data might be useful as well for grouping of the chemicals and we aimed for investigating this.

Datamining of (Q)SAR predictions is such a potential tool, and we aimed to evaluate the usefulness of this tool for grouping purposes.

4.2 Aim

We wanted to make a small preliminary investigation as to whether data mining of (Q)SAR predictions can be used to group 20 PFAS according to one or more adverse effects, environmental fate and/or physico-chemical parameters.

Our aim was to investigate whether we can use (Q)SAR modeling for allocating a new PFAS to a specific group of PFAS. In some cases we may have a mixture containing a specific perfluorinated carboxylic acid with limited hazard data and we asked whether QSAR modeling can help us telling whether it is reasonable or not to group this compound together with other PFCAs. This same question can be asked for fluorotelomer alcohols and perfluorinated sulphonates.

4.3 Method

- (Q)SAR predictions were retrieved from the Danish (Q)SAR Database (http://qsar.food.dtu.dk/) for nine PFCAs (perfluoroalkyl carboxylic acids, C4-C12), eight PFSAs (perfluoroalkane sulfonic acids, C4-C12) and three n:2 FTOHs (fluorotelomer alcohols, C4, C6 and C8) and a report generated for each of them (Oct 2018).
- We selected several relevant predicted endpoints (adverse effects, environmental fate and physico-chemical parameters) and prepared an overview of the predicted parameters.
- We investigated whether there is a pattern in the predicted parameters that may allow us to group the chemicals.
- Two approaches were used:
    1) One data mining approach based on an open question to **Leadscope Predictive Data Miner version 3.5.3 (http://www.leadscope.com/)**
    2) A decision tree modeling approach using **WEKA** version 3.8 (freeware), where the system was asked if it could make a small model to classify the compounds into the three chemical classes (carboxylic acids, sulphonates and alcohols) based on the QSAR-predicted parameters as descriptors.

Table 6: Overview of system settings in Leadscope

Name for this run: Clusters_PF_2019_pred_only_2 Project: PF_2019 # structures: 20 Analysis type: Clustering (Agglomerative Nesting) Date: Fri Feb 01 14:01:38 CET 2019 Owner: nign Create signatures: true Group singletons: true Cluster by: Data (PF_Allergic-Contact-Dermatitis PF_Ames-battery PF_AR-anta-battery PF_Ashby-battery PF_Base-Pair-Ames-battery PF_BCF(Arnot-Gobas-incl-biotransf) PF_BCF(L-kg-wet-wt) PF_Biowin1 PF_Biowin2 PF_Biowin3 PF_Biowin4 PF_Biowin5 PF_Biowin6 PF_Biowin7 PF_CA-CHL-battery PF_CA-CHO-battery PF_CU-Carc-FM PF_CU-Carc-FR PF_CU-Carc-M PF_CU-Carc-MM PF_CU-Carc-MR PF_CU-Carc-Rat PF_CU-Carc-Rodent PF_Direct-act-mutagens-battery PF_EC50(algae96h) PF_EC50(daphnid48h) PF_EC50-Algae-battery(mg/L) PF_EC50-Daph-battery(mg/L) PF_ER-a-binding-battery(balanced) PF_Frameshift-Ames-battery PF_GI-abs(1mg) PF_LC50(fish96h) PF_LC50-Fish-battery(mg/L) PF_LipinskisRule PF_Liver-spec-cancer-Rodent-battery PF_LogBBB PF_LogD(7) PF_LogKaw PF_LogKoa PF_LogKoc(Kow) PF_LogKoc(MCI) PF_LogKow PF_LS-Carc-FM PF_LS-Carc-FR PF_LS-Carc-M PF_LS-Carc-MM PF_LS-Carc-MR PF_LS-Carc-Rat PF_LS-Carc-Rodent PF_Mut-HGPRT-battery PF_Mut-Thymid-Kinase-Locus-battery PF_NotReadyBiodeg(battery) PF_Persist-time(days) PF_Phi(Mackay) PF_pKa PF_Potent-Ames-battery PF_PXR-binding-battery PF_Rat-oral-acute(LD50_mg-kg-d) PF_SCE-vivo-battery PF_SHE-cell-trans-battery PF_t-half-air PF_t-half-sedi PF_t-half-soil PF_t-half-water PF_WS(Kow)) Linkage mechanism: Average Linkage Cluster height: 0.5

4.4 Results

Table 7: The QSAR predictions for the 20 PFAS that the clustering exercise is based upon (obtained from the Danish QSAR database)

	Chemical name	Registry No	Class	Water. Sol from Kow (mg/L)	Log Kow	pKa Acid	LogD (7)	LogKoa	LogKaw	Phi-Mackay-based	Log Koc (MCI)	Log Koc (Kow)	t½ air	t½ water	t½ soil	t½ sedi	Persistence time (days)	Biowin1	Biowin2	Biowin3	Biowin4	Biowin5
1	3,3,4,4,5,5,6,6,6-nonafluorohexanol	2043-47-2	Fl. Alcohol	9,70E+01	3,07	13,5	3,09	3,954	-0,654	1,96E-07	2,8323	2,339	61,4	4320	8640	38900	9,17	-0,2917	0	1,6262	2,861	0,5457
2	3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctan-1-ol	647-42-7	Fl. Alcohol	2,19E+00	4,41	13,5	4,53	3,752	0,788	2,09E-06	4,1355	3,025	61,4	4320	8640	38900	12,33	-0,7071	0	0,981	2,410	0,4529
3	3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluorodecan-1-ol	678-39-7	Fl. Alcohol	6,74E-02	5,75	13,5	5,87	3,351	2,229	2,07E-05	5,4388	3,600	61,4	4320	8640	38900	95,00	-1,1226	0	0,3357	1,959	0,3601
4	heptafluorobutyric acid	375-22-4	Carbox. Acid	1,37E+03	2,14	0,6	-2,34	4,453	-2,313	2,83E-07	1,8126	1,339	494	1440	2880	13000	30,96	-0,1699	0,0002	2,1536	3,343	0,5134
5	sodium perfluorovalerate	2706-89-0	Carbox. Acid	1,97E+02	2,81	0,6	-1,72	4,402	-1,592	3,81E-07	2,4642	1,710	494	1440	2880	13000	29,33	-0,3777	0	1,8309	3,118	0,467
6	undecafluorohexanoic acid	307-24-4	Carbox. Acid	2,71E+01	3,48	0,6	-1	4,351	-0,871	1,00E-06	3,1158	2,081	494	4320	8640	38900	34,75	-0,5854	0	1,5083	2,892	0,4206
7	perfluoroheptanoic acid	375-85-9	Carbox. Acid	3,65E+00	4,15	2,4	-0,25	4,300	-0,150	1,35E-05	3,7674	2,451	494	4320	8640	38900	33,33	-0,7932	0	1,1857	2,667	0,3742
8	pentadecafluorooctanoic acid	335-67-1	Carbox. Acid	4,81E-01	4,81	2,4	0,62	4,240	0,570	1,73E-06	4,4191	2,816	494	4320	8640	38900	39,63	-1,0009	0	0,8631	2,441	0,3278
9	perfluorononan-1-oic acid	375-95-1	Carbox. Acid	6,26E-02	5,48	2,4	1,32	4,190	1,290	1,62E-05	5,0707	3,187	494	4320	8640	38900	72,08	-1,2086	0	0,5404	2,215	0,2814
10	nonadecafluorodecanoic acid	335-76-2	Carbox. Acid	8,04E-03	6,15	2,4	1,87	4,139	2,011	3,91E-05	5,7223	3,558	494	4320	8640	38900	152,92	-1,4164	0	0,2178	1,990	0,235
11	ammonium octadecafluoro-9-(trifluoromethyl)decanoate	3658-63-7	Carbox. Acid	1,02E-03	6,82	2,4	2,58	4,088	2,732	9,14E-05	6,3739	3,928	494	4320	8640	38900	235,00	-1,6241	0	-0,1048	1,764	0,1886
12	tricosafluorododecanoic acid	307-55-1	Carbox. Acid	1,29E-04	7,49	2,4	3,24	4,038	3,452	6,43E-05	7,0256	4,299	494	4320	8640	38900	269,17	-1,8318	0	-0,4275	1,539	0,1422
13	1,1,2,2,3,3,4,4,4-nonafluorobutane-1-sulphonic acid	375-73-5	Sulfonates	3,44E+02	1,82	-5,8	-4,2	5,050	-3,230	2,72E-05	2,2489	1,932	1830	4320	8640	38900	106,25	-0,3592	0,0052	1,5793	2,857	0,1786
14	perfluoropentane-1-sulphonic acid	2706-91-4	Sulfonates	4,66E+01	2,49	-5,8	-3,44	5,000	-2,510	1,22E-04	2,9005	2,302	1830	4320	8640	38900	137,08	-0,5669	0,0005	1,2566	2,632	0,1322
15	perfluorohexane-1-sulphonic acid	355-46-4	Sulfonates	6,17E+00	3,16	-5,8	-2,75	4,950	-1,790	2,80E-04	3,5522	2,673	1830	4320	8640	38900	145,83	-0,7746	0	0,934	2,406	0,0858
16	1,1,2,2,3,3,4,4,5,5,6,6,7,7,7-pentadecafluoroheptane-1-sulphonic acid	375-92-8	Sulfonates	8,06E-01	3,82	-5,7	-2,19	4,888	-1,068	1,28E-04	4,2038	3,038	1830	4320	8640	38900	149,58	-0,9824	0	0,6114	2,180	0,0394
17	heptadecafluorooctane-1-sulphonic acid	1763-23-1	Sulfonates	1,04E-01	4,49	-5,7	-1,51	4,837	-0,347	1,59E-04	4,8554	3,409	1830	4320	8640	38900	168,33	-1,1901	0	0,2887	1,955	-0,007
18	ammonium nonadecafluorononanesulphonate	17202-41-4	Sulfonates	1,33E-02	5,16	-5,7	-0,82	4,787	0,373	1,98E-04	5,507	3,779	1830	4320	8640	38900	231,25	-1,3978	0	-0,0339	1,729	-0,0534
19	henicosafluorodecanesulphonic acid	335-77-3	Sulfonates	1,68E-03	5,83	-5,7	-0,15	4,737	1,093	2,47E-04	6,1587	4,150	1830	4320	8640	38900	321,25	-1,6056	0	-0,3565	1,504	-0,0998
20	1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,12-pentacosafluorododecar	79780-39-5	Sulfonates	2,64E-05	7,17	-5,7	1,3	4,636	2,534	3,82E-04	7,4619	4,891	1830	4320	8640	38900	377,92	-2,0211	0	-1,0018	1,053	-0,1926

	Chemical name	Biowin6	Biowin7	Not Ready biodeg (battery)	BCF (L/kg wet-wt)	BCF (Arnot-Gobas incl biotransf)	Fathead minnow LC50, battery	Daphnia magna EC50, battery	Pseudokirchneriella s. EC50, battery	LC50 (fish 96h)	EC50 (daphnid 48h)	EC50 (algae 96h)	Lipinski's Rule	Absorp. from gast. Intest. Tract for 1 mg dose (%)	Log brain/blood part coeff	Rat oral (LD50 mg/kg/d)
1	3,3,4,4,5,5,6,6,6-nonafluorohexanol	0	0,0181	POS_IN	69,88	159	1788	69,5276	38,9389	23,74	14,9	16,68	0	100	0,476	790
2	3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctan-1-ol	0	-0,6503	INC_OUT	459,70	1511	28249	229,9173	14,1012	2,06	1,5	2,72	0	100	0,744	830
3	3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluorodecan-1-ol	0	-1,3188	POS_OUT	2232,00	6124	96427	34,2668	3,3012	0,17	0,1	0,41	1	100	1,012	570
4	heptafluorobutyric acid	0	0,3544	POS_OUT	3,16	15	1084	29,8883	248,1984	1322,59	760,6	597,14	0	95	0,119	760
5	sodium perfluorovalerate	0	0,0202	INC_OUT	3,16	65	3920	43,0372	170,1420	408,97	250,2	253,58	0	95	0,253	540
6	undecafluorohexanoic acid	0	-0,3141	INC_OUT	3,16	280	10900	33,1823	106,4873	121,93	79,3	103,82	0	100	0,387	460
7	perfluoroheptanoic acid	0	-0,6483	INC_OUT	3,16	1119	27357	20,7623	65,2647	35,43	24,5	41,43	0	100	0,521	400
8	pentadecafluorooctanoic acid	0	-0,9825	POS_OUT	3,16	3749	133766	37,2093	30,3937	10,10	7,4	16,22	0	100	0,653	420
9	perfluorononan-1-oic acid	0	-1,3168	POS_OUT	10,00	9131	100940	2,7439	19,5767	2,84	2,2	6,26	1	100	0,787	390
10	nonadecafluorodecanoic acid	0	-1,6510	POS_OUT	56,23	13710	Domain OUT	1,4040	14,1700	0,79	0,7	2,39	2	100	0,921	240
11	ammonium octadecafluoro-9-(trifluoromethyl)decanoate	0	-1,9852	POS_OUT	56,23	10950	Domain OUT	0,8678	7,9056	0,22	0,2	0,90	2	100	1,055	240
12	tricosafluorododecanoic acid	0	-2,3194	POS_OUT	56,23	5385	Domain OUT	Domain OUT	4,8961	0,06	0,1	0,34	2	95	1,189	230
13	1,1,2,2,3,3,4,4,4-nonafluorobutane-1-sulphonic acid	0	-0,1666	POS_OUT	3,16	7	Domain OUT	133,1333	Domain OUT	3597,02	2008,2	1395, 165	0	90	-0,116	960
14	perfluoropentane-1-sulphonic acid	0	-0,5008	POS_OUT	3,16	31	Domain OUT	123,3222	Domain OUT	1051,87	624,7	560,29	0	95	0,018	1000
15	perfluorohexane-1-sulphonic acid	0	-0,8351	POS_OUT	3,16	129	114617	49,0486	Domain OUT	301,32	190,4	220,42	0	100	0,152	1000
16	1,1,2,2,3,3,4,4,5,5,6,6,7,7,7-pentadecafluoroheptane-1-sulphonic acid	0	-1,1693	POS_OUT	3,16	508	159330	320,3687	Domain OUT	84,97	57,1	85,36	0	100	0,284	1400
17	heptadecafluorooctane-1-sulphonic acid	0	-1,5035	POS_OUT	3,16	1715	177032	18,2046	51,9475	23,66	16,9	32,65	1	95	0,418	1500
18	ammonium nonadecafluorononanesulphonate	0	-1,8378	POS_OUT	10,00	4600	194735	15,8669	27,2491	6,53	5,0	12,36	2	95	0,552	1100
19	henicosafluorodecanesulphonic acid	0	-2,1720	POS_OUT	10,00	8884	212433	12,1326	13,9966	1,78	1,4	4,64	2	95	0,686	1200
20	1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,12-pentacosafluorododecan	0	-2,8404	POS_OUT	56,23	6575	Domain OUT	Domain OUT	3,8300	0,13	0,1	0,64	2	90	0,954	1300

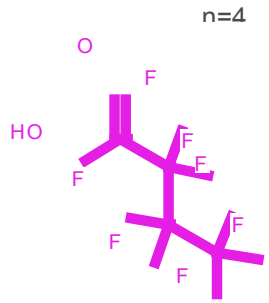
	Chemical name	Allergic contact derma in GP and human	Resp sensitisation in humans	ERa binding, battery (full training set)	Era binding, battery (balanced training set)	ERa activation, battery	AR anta, battery	TRa binding, battery	TRb binding, battery	PXR binding, battery	Teratogenic potential in humans, battery	Ashby structural alerts, battery	Ames test, battery	Direct acting mutagens (without S9), battery	Base-Pair Ames Mutagens, battery	Frameshift Ames Mutagens, battery
1	3,3,4,4,5,5,6,6,6-nonafluorohexanol	INC_OUT	INC_OUT	POS_OUT	POS_IN	NEG_OUT	NEG_IN			NEG_IN	NEG_OUT	NEG_IN	NEG_IN	INC_OUT	POS_IN	NEG_IN
2	3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctan-1-ol	INC_OUT	POS_OUT	POS_OUT	POS_IN	NEG_OUT	NEG_IN			NEG_IN	INC_OUT	NEG_IN	NEG_IN	INC_OUT	POS_IN	NEG_IN
3	3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluorodecan-1-ol	INC_OUT	INC_OUT	POS_OUT	POS_IN	NEG_OUT	NEG_IN			NEG_IN	INC_OUT	NEG_IN	NEG_IN	NEG_OUT	POS_IN	NEG_IN
4	heptafluorobutyric acid	POS_IN	INC_OUT	INC_OUT	NEG_IN	NEG_OUT	NEG_IN			NEG_IN	NEG_OUT	INC_OUT	NEG_IN	POS_IN	POS_OUT	NEG_IN
5	sodium perfluorovalerate	POS_IN	INC_OUT	INC_OUT	NEG_IN	NEG_OUT	NEG_IN			NEG_IN	NEG_OUT	NEG_OUT	NEG_IN	POS_IN	POS_OUT	NEG_IN
6	undecafluorohexanoic acid	POS_IN	INC_OUT	INC_OUT	INC_OUT	NEG_OUT	NEG_IN			NEG_IN	NEG_OUT	NEG_IN	NEG_IN	POS_IN	POS_OUT	NEG_IN
7	perfluoroheptanoic acid	POS_IN	INC_OUT	INC_OUT	INC_OUT	NEG_OUT	NEG_IN			NEG_IN	NEG_OUT	NEG_IN	NEG_IN	POS_OUT	POS_IN	NEG_IN
8	pentadecafluorooctanoic acid	POS_IN	INC_OUT	INC_OUT	INC_OUT	NEG_OUT	NEG_IN			NEG_IN	NEG_OUT	NEG_IN	NEG_IN	POS_OUT	POS_IN	NEG_IN
9	perfluorononan-1-oic acid	POS_IN	INC_OUT	INC_OUT	POS_IN	NEG_OUT	NEG_IN			NEG_IN	NEG_OUT	NEG_IN	NEG_IN	INC_OUT	POS_IN	NEG_IN
10	nonadecafluorodecanoic acid	POS_IN	INC_OUT	POS_OUT	POS_IN	NEG_OUT	NEG_IN			NEG_IN	NEG_OUT	NEG_IN	NEG_IN	INC_OUT	POS_IN	NEG_IN
11	ammonium octadecafluoro-9-(trifluoromethyl)decanoate	POS_IN	INC_OUT	POS_OUT	POS_IN	NEG_OUT	NEG_IN			INC_OUT	NEG_OUT	NEG_IN	NEG_IN	NEG_OUT	POS_IN	NEG_IN
12	tricosafluorododecanoic acid	POS_IN	INC_OUT	POS_OUT	POS_IN	NEG_OUT	NEG_IN			INC_OUT	NEG_OUT	NEG_IN	NEG_IN	NEG_OUT	POS_IN	NEG_IN
13	1,1,2,2,3,3,4,4,4-nonafluorobutane-1-sulphonic acid	POS_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	NEG_IN			NEG_IN	INC_OUT	NEG_OUT	NEG_IN	INC_OUT	INC_OUT	INC_OUT
14	perfluoropentane-1-sulphonic acid	POS_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	NEG_IN			NEG_OUT	INC_OUT	NEG_IN	NEG_IN	INC_OUT	INC_OUT	INC_OUT
15	perfluorohexane-1-sulphonic acid	POS_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	NEG_IN			NEG_OUT	INC_OUT	NEG_IN	NEG_IN	INC_OUT	INC_OUT	INC_OUT
16	1,1,2,2,3,3,4,4,5,5,6,6,7,7,7-pentadecafluoroheptane-1-sulphonic acid	POS_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	NEG_IN			NEG_OUT	INC_OUT	NEG_IN	NEG_IN	INC_OUT	INC_OUT	INC_OUT
17	heptadecafluorooctane-1-sulphonic acid	POS_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	NEG_IN			INC_OUT	INC_OUT	NEG_IN	NEG_IN	INC_OUT	INC_OUT	INC_OUT
18	ammonium nonadecafluorononanesulphonate	POS_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	NEG_IN			INC_OUT	INC_OUT	NEG_IN	NEG_OUT	INC_OUT	INC_OUT	INC_OUT
19	henicosafluorodecanesulphonic acid	POS_IN	INC_OUT	INC_OUT	INC_OUT	INC_OUT	NEG_IN			INC_OUT	INC_OUT	NEG_IN	NEG_OUT	INC_OUT	INC_OUT	INC_OUT
20	1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,12-pentacosafluorododecar	POS_IN	INC_OUT	INC_OUT	INC_OUT	INC_OUT	NEG_IN			INC_OUT	INC_OUT	NEG_IN	INC_OUT	INC_OUT	INC_OUT	INC_OUT

	Chemical name	Potent Ames Mutagens, battery	Chromosome aberr CHO cells, battery	Chromosome aberr CHL, battery	Mutations in Thymidine Kinase Locus, battery	Mutations in HGPRT locus (CHO), battery	Unscheduled DNA synt (UDS), battery	Syrian Hamster embryo cell transformation, battery	SLRL, battery	Micronucleus, battery	Dominant lethal mutation, battery	Sister chromatide exchange, battery	Comet assay mouse, battery	FDA Male rat,	FDA Female rat
1	3,3,4,4,5,5,6,6,6-nonafluorohexanol	INC_OUT	NEG_IN	NEG_IN	POS_OUT	NEG_IN	INC_OUT	NEG_OUT	NEG_OUT	INC_OUT	INC_OUT	POS_IN	INC_OUT	POS_OUT	NEG_IN
2	3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctan-1-ol	POS_OUT	NEG_IN	NEG_IN	NEG_OUT	NEG_IN	INC_OUT	NEG_OUT	NEG_OUT	INC_OUT	INC_OUT	POS_IN	INC_OUT	POS_OUT	NEG_IN
3	3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluorodecan-1-ol	POS_IN	NEG_IN	NEG_IN	INC_OUT	NEG_IN	INC_OUT	NEG_IN	NEG_OUT	INC_OUT	INC_OUT	POS_OUT	INC_OUT	POS_OUT	NEG_IN
4	heptafluorobutyric acid	POS_OUT	NEG_IN	NEG_IN	NEG_OUT	NEG_IN	INC_OUT	NEG_IN	NEG_OUT	INC_OUT	NEG_OUT	POS_OUT	INC_OUT	POS_OUT	NEG_IN
5	sodium perfluorovalerate	POS_OUT	NEG_IN	NEG_IN	NEG_OUT	NEG_IN	INC_OUT	NEG_IN	NEG_OUT	INC_OUT	INC_OUT	POS_OUT	INC_OUT	POS_OUT	NEG_IN
6	undecafluorohexanoic acid	POS_IN	NEG_IN	NEG_IN	NEG_IN	NEG_IN	INC_OUT	NEG_IN	NEG_OUT	INC_OUT	INC_OUT	POS_OUT	INC_OUT	POS_OUT	NEG_IN
7	perfluoroheptanoic acid	POS_IN	NEG_IN	NEG_IN	NEG_OUT	NEG_IN	INC_OUT	NEG_IN	NEG_OUT	INC_OUT	INC_OUT	POS_OUT	INC_OUT	POS_OUT	NEG_IN
8	pentadecafluorooctanoic acid	POS_IN	NEG_IN	NEG_IN	NEG_OUT	NEG_IN	INC_OUT	NEG_IN	NEG_OUT	INC_OUT	INC_OUT	POS_OUT	INC_OUT	POS_IN	NEG_IN
9	perfluorononan-1-oic acid	POS_IN	NEG_IN	NEG_IN	NEG_OUT	NEG_IN	INC_OUT	NEG_IN	NEG_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	POS_OUT	NEG_IN
10	nonadecafluorodecanoic acid	POS_IN	NEG_IN	NEG_IN	NEG_OUT	NEG_IN	INC_OUT	NEG_IN	NEG_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	POS_OUT	NEG_IN
11	ammonium octadecafluoro-9-(trifluoromethyl)decanoate	POS_IN	NEG_IN	NEG_IN	NEG_OUT	NEG_IN	INC_OUT	NEG_IN	NEG_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	POS_OUT	NEG_IN
12	tricosafluorododecanoic acid	POS_IN	NEG_IN	NEG_IN	NEG_OUT	NEG_IN	INC_OUT	NEG_IN	NEG_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	POS_OUT	NEG_IN
13	1,1,2,2,3,3,4,4,4-nonafluorobutane-1-sulphonic acid	INC_OUT	NEG_IN	NEG_IN	INC_OUT	NEG_OUT	INC_OUT	INC_OUT	NEG_OUT	INC_OUT	NEG_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT
14	perfluoropentane-1-sulphonic acid	INC_OUT	NEG_IN	NEG_OUT	INC_OUT	NEG_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT
15	perfluorohexane-1-sulphonic acid	INC_OUT	NEG_IN	NEG_OUT	INC_OUT	NEG_IN	INC_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT
16	1,1,2,2,3,3,4,4,5,5,6,6,7,7,7-pentadecafluoroheptane-1-sulphonic acid	INC_OUT	NEG_IN	NEG_OUT	INC_OUT	NEG_IN	INC_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT
17	heptadecafluorooctane-1-sulphonic acid	INC_OUT	NEG_IN	NEG_IN	INC_OUT	NEG_IN	INC_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT
18	ammonium nonadecafluorononanesulphonate	INC_OUT	NEG_IN	NEG_IN	INC_OUT	NEG_IN	INC_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT
19	henicosafluorodecanesulphonic acid	INC_OUT	NEG_IN	NEG_IN	INC_OUT	NEG_IN	INC_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT
20	1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,12-pentacosafluorododecar	INC_OUT	NEG_IN	NEG_IN	INC_OUT	NEG_IN	INC_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT

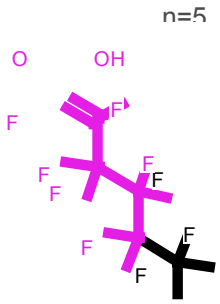
	Chemical name	FDA Rat	FDA male mouse	FDA female mouse	FDA mouse	FDA rodent	FDA Male rat,	FDA Female rat	FDA Rat	FDA male mouse	FDA female mouse	FDA mouse	FDA rodent	Liver specific cancer in rat or mouse, battery
1	3,3,4,4,5,5,6,6,6-nonafluorohexanol	NEG_IN	NEG_IN	NEG_IN	NEG_IN	NEG_IN	INC_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	NEG_OUT	INC_OUT	NEG_IN
2	3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctan-1-ol	NEG_IN	NEG_IN	NEG_IN	NEG_IN	NEG_IN	INC_OUT	NEG_IN	INC_OUT	NEG_IN	NEG_OUT	NEG_IN	INC_OUT	NEG_IN
3	3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluorodecan-1-ol	NEG_IN	NEG_IN	NEG_IN	NEG_IN	NEG_IN	INC_OUT	NEG_IN	INC_OUT	NEG_IN	NEG_IN	NEG_IN	INC_OUT	NEG_IN
4	heptafluorobutyric acid	POS_OUT	NEG_IN	NEG_IN	NEG_IN	POS_OUT	NEG_OUT	NEG_IN	NEG_IN	NEG_IN	NEG_OUT	NEG_IN	NEG_OUT	NEG_OUT
5	sodium perfluorovalerate	POS_OUT	NEG_IN	NEG_IN	NEG_IN	POS_OUT	NEG_IN	NEG_IN	NEG_IN	NEG_IN	NEG_IN	NEG_IN	NEG_IN	NEG_OUT
6	undecafluorohexanoic acid	POS_OUT	NEG_IN	NEG_IN	NEG_IN	POS_OUT	NEG_IN	NEG_IN	NEG_IN	NEG_IN	NEG_IN	NEG_IN	NEG_IN	INC_OUT
7	perfluoroheptanoic acid	POS_OUT	NEG_IN	NEG_IN	NEG_IN	POS_OUT	NEG_IN	NEG_IN	NEG_IN	NEG_IN	NEG_IN	NEG_IN	NEG_IN	INC_OUT
8	pentadecafluorooctanoic acid	POS_IN	NEG_IN	NEG_IN	NEG_IN	POS_IN	NEG_IN	NEG_IN	NEG_IN	NEG_IN	NEG_IN	NEG_IN	NEG_IN	INC_OUT
9	perfluorononan-1-oic acid	POS_OUT	NEG_IN	NEG_IN	NEG_IN	POS_OUT	NEG_IN	NEG_IN	NEG_IN	NEG_IN	NEG_IN	NEG_IN	NEG_IN	INC_OUT
10	nonadecafluorodecanoic acid	POS_OUT	NEG_IN	NEG_IN	NEG_IN	POS_OUT	NEG_IN	NEG_IN	NEG_IN	NEG_IN	NEG_IN	NEG_IN	NEG_IN	INC_OUT
11	ammonium octadecafluoro-9-(trifluoromethyl)decanoate	POS_OUT	NEG_IN	NEG_IN	NEG_IN	POS_OUT	NEG_IN	NEG_IN	NEG_IN	NEG_IN	NEG_IN	NEG_IN	NEG_IN	INC_OUT
12	tricosafluorododecanoic acid	POS_OUT	NEG_IN	NEG_IN	NEG_IN	POS_OUT	NEG_IN	NEG_IN	NEG_IN	NEG_IN	NEG_IN	NEG_IN	NEG_IN	INC_OUT
13	1,1,2,2,3,3,4,4,4-nonafluorobutane-1-sulphonic acid	INC_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	NEG_OUT	NEG_OUT	INC_OUT	POS_OUT	INC_OUT	NEG_OUT
14	perfluoropentane-1-sulphonic acid	INC_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	NEG_OUT	NEG_OUT	INC_OUT	POS_OUT	INC_OUT	NEG_OUT
15	perfluorohexane-1-sulphonic acid	INC_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	NEG_OUT	NEG_OUT	NEG_OUT	POS_OUT	INC_OUT	NEG_OUT
16	1,1,2,2,3,3,4,4,5,5,6,6,7,7,7-pentadecafluoroheptane-1-sulphonic acid	INC_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	NEG_OUT	NEG_OUT	NEG_OUT	INC_OUT	INC_OUT	NEG_OUT
17	heptadecafluorooctane-1-sulphonic acid	INC_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	NEG_OUT	NEG_IN	NEG_IN	INC_OUT	INC_OUT	NEG_OUT
18	ammonium nonadecafluorononanesulphonate	INC_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	NEG_OUT	NEG_IN	NEG_IN	NEG_OUT	INC_OUT	NEG_OUT
19	henicosafluorodecanesulphonic acid	INC_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	NEG_OUT	NEG_IN	NEG_IN	NEG_OUT	INC_OUT	NEG_OUT
20	1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,12-pentacosafluorododecar	INC_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	INC_OUT	NEG_OUT	NEG_IN	NEG_IN	NEG_OUT	INC_OUT	INC_OUT

4.4.1 Data mining of QSAR predictions in Leadscope

Cluster 1 – PFxA with n=4-5:

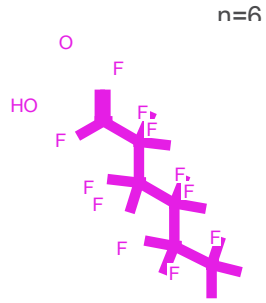


PF_2019_375-22-4

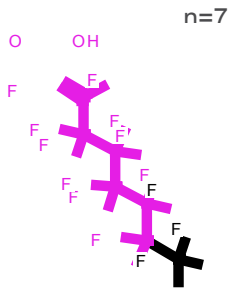


PF_2019_2706-89-0

Cluster 2 – PFxA with n=6-7:

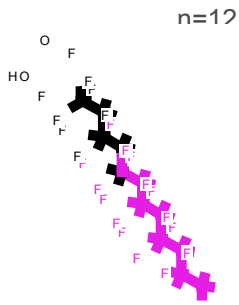


PF_2019_307-24-4

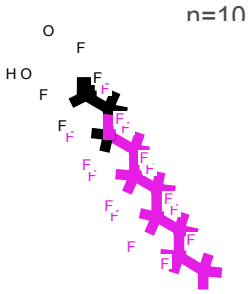


PF_2019_375-85-9

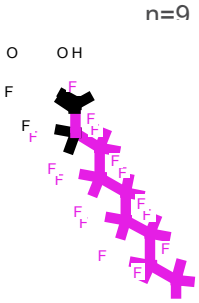
Cluster 3 – PFxA with n=9-12:



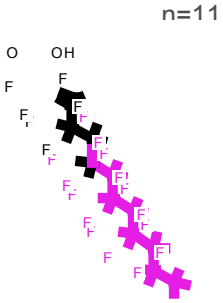
PF_2019_307-55-1



PF_2019_335-76-2

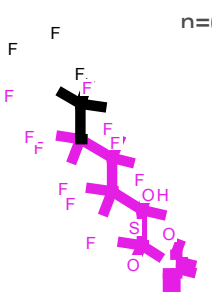


PF_2019_375-95-1

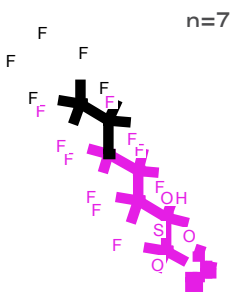


PF_2019_3658-63-7

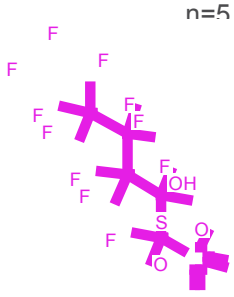
Cluster 4 – PFxS with n=5-7:



PF_2019_355-46-4

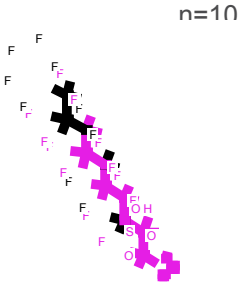


PF_2019_375-92-8

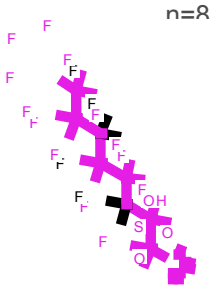


PF_2019_2706-91-4

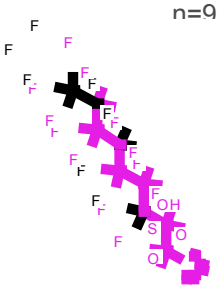
Cluster 5 – PFxS with n=8-12:



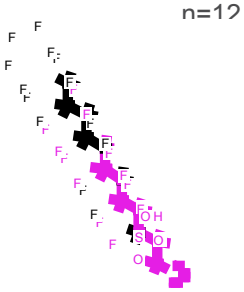
PF_2019_335-77-3



PF_2019_1763-23-1

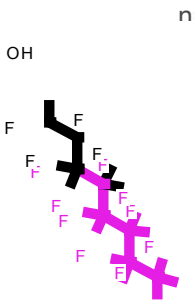


PF_2019_17202-41-4

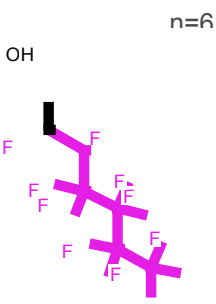


PF_2019_79780-39-5

Cluster 6 – FtOH with n=6-8:



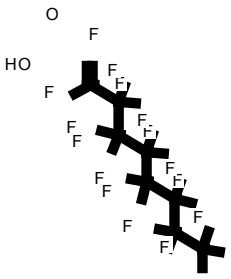
PF_2019_647-42-7



PF_2019_2043-47-2

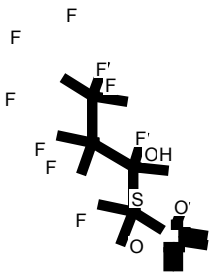
Singletons not belonging to any group:

PFxA with



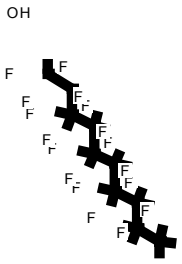
PF_2019_335-67-1

PFxS with



PF_2019_375-73-5

FtOH with



PF_2019_678-39-7

Leadscope was asked the open question, if the system could group these 20 PFAS into clusters based on the QSAR predictions. The system was set to cluster by data (and not by chemical structures) by using the default setting. Only descriptors that had at least one prediction within the domain were imported into the system.

It provided the results in Table 8 with 6 clusters and 3 so-called singletons.

Table 8: The clustering output from Leadscope for PFCAs, PFSAs and FtOHs

	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6	Singleton
PFCA	N=4-5	N=6-7	N=9-12				N=8
PFSA				N=5-7	N=8-12		N=4
FtOH						N=6 & 8	N=10

The clustering depended in most cases on the chain length. For the PFCAs, PFBA and PFPA were grouped together in one cluster, PFHxA and PFHpA were grouped in another cluster and the longer chained PFNA, PFDA, PFUnDA, and PFDDA were grouped together in a third cluster.

The exception was PFOA, which to our surprise was a singleton in this preliminary exercise, and not grouped together with any other PFCA.

For the PFSAs, the compounds with chain length of 5 to 7 were grouped together, as was those with chain length 8 to 12. However, the short-chained PFBS was clustered as a singleton.

For the FtOHs, the two short chained were grouped together (N= 6 & 8), whereas N=10 was a singleton.

4.4.2 Decision tree modeling based on QSAR predictions in WEKA

We investigated whether the (Q)SAR predictions can be used to predict which of the three classes the PFASs belong to by developing a so-called decision tree in WEKA version 3.8.

With this system, no open question was asked, but rather the system was asked to generate decision rules for classification of the 20 compounds into the three chemical classes, the PFCAs, the PFSAs or the FtOHs.

A number of simple decision trees using a variation of many different descriptors among the available QSAR-predictions, could be generated, all of them achieving internal classification precision of 100% on the training set of 20 compounds. For example, based on Frameshift (negative) and half-life in air (two different cut-off values for the acids and the sulfonates), the model could discriminate its training set 100% in terms of substance class. However, this is not validated, and since it is a very small model, it is of course uncertain.

In the decision tree, the most discriminating descriptor was the half-life in air (hours) as predicted by the EPI Level III Fugacity Model (EPI Suite v4.11). This is due to an artefact as the substances in each of the three classes have the same value that are unique for the specific class. This is probably due to the way the model works to calculate rate constants for the atmospheric reactions (in EPI Aopwin), which is based on defined fragment and reaction values.

Excluding this descriptor forces the system to choose others. By using this approach, the common feature of all other descriptors, which the system chose for this model, was that the lack of predictions (due to out-of-domain) for one or more of the three classes was crucial for the discrimination. This obviously indicates a weakness in this very small and preliminary investigation, because if the QSAR model domain was enlarged, this discrimination may disappear. Below is shown an example of a generated decision tree. It is just one of many options for decision trees that can be made due to the dataset (training set predicted with 100% accuracy, and external accuracy examined with leave-one-out cross-validation gave 80%, that is 4 misclassified, of which 2 were alcohols, 1 carboxylic acid and 1 sulfonic acid, which means that the alcohol class having only three members was as expected weakest).

One out of many examples of decision trees:

IF FDA_Rat (cancer) = NEG_IN THEN Class = acid
ELSE
IF pKa_Acid <= 2.4 THEN Class = sulfonates
ELSE
Class = alcohol (3.0)

4.5 Conclusion on QSAR predictions

WEKA came out with very many classification models, but no one was outstanding and seemed to be much better than the others. WEKA suggested that all perfluorinated carboxylic acids can be grouped together independent of chain length. Similarly, all perfluorinated sulphonates can be grouped in another group and the fluortelomers in a third group.

In contrast, Leadscope suggested a more fine-tuned clustering that was very dependent both on the chain length and the identity of the head group for each of the chemical classes.

Overall, we have to be cautious with the conclusions due to the very low number of chemical structures included in this analysis. In addition, PFAS were in many cases outside the

applicability domain of the QSAR model, which hinders a good prediction for these endpoints. In order to follow up on this, we need a larger number of chemicals included in the analysis.

To sum it up, the preliminary studies that we have made indicate that the QSAR predictions can be used to some extent to predict whether a PFAS belongs to one of the three structural classes.

5 HBM data together with in vitro data for informing risk assessment of PFOS

New ways of risk assessing chemicals are needed, and we investigated whether it is possible – based on the current information level - to evaluate human risk to selected thyroid toxicants by use of non-animal data and HBM data. The use of human-based in vitro data and HBM data has the obvious advantage of avoiding the well-known species-specific differences that are evident for many chemicals. Combining such human-based hazard outputs with actual measured human exposure data has the potential to elucidate the real human risk to chemical exposure.

5.1 Methodology

PFOS was selected for investigation based on its known thyroid toxic effect in vivo and relevant human exposure as well as four other thyroid toxicants for comparison. A literature search was conducted to extract HBM data (NHANES and PubMed) and thyroid relevant in vitro data (PubMed and ToxCast database). For comparison we also included animal in vivo studies from which NOAELs for thyroid effects were derived. To enable comparison across HBM, in vitro and in vivo studies, chemical concentrations in human and animal blood was transformed to nM. Briefly, chemical blood levels from epidemiological and in vivo studies were re-calculated from the unit g chemical/ g lipid or g chemical/ g wet weight of blood to nM. Blood levels were estimated by calculation of daily intake based on concentrations found in urine and hereafter a simplified one-compartment toxicokinetic model was applied.

A risk characterization ratio (RCR) was calculated for each chemical by division of the exposure estimate based on HBM data with a reference value (RV) based on in vitro data (RCR=exposure/RV). The RCR value reflects whether exposures exceed the concentrations considered “safe”. Thus, a RCR value >1 indicate that human exposure levels may be associated with a potential risk. The RV was based on in vitro data from one experimental study for each chemical that was selected based on expert judgement in terms of relevance of the mechanism of action and reliability of the study.

The in vitro studies included are presented in the table below. The data shows a wide field of tested in vitro endpoints such as thyroid peroxidase inhibition, antagonism of the thyroid receptor (TR) and transthyretin (TTR) binding by PFOS.

To take uncertainty of HBM data into account the exposure values were calculated based on an average of means. Exposure data with values below limit of quantification/limit of detection, measurements in other matrices than blood or urine, studies from Asian countries and occupational exposure studies, were excluded.

Our approach and results are described in detail in Johansson et al., 2019.

Table 1 Case study literature overview for the five selected chemicals PFOS, triclosan, TBBPA, BDE-209 and HBCD. The first row shows the human biomonitoring (HBM) data with country of origin and references. The second row shows the *in vitro* data with end points measured, LOEC/EC_x values, the study used for calculation of risk characterization ratio (RCR) marked in bold and references. The third row shows the *in vivo* data from animal experiments with end points and references.

Data type	PFOS	Triclosan	TBBPA	BDE-209	HBCD
HBM data (country of origin)	US [22] Denmark [36]	US [22] Denmark [55]	France [56,57] Belgium [58]	US [59,60] UK [61–63] Greece [64] Spain [63,65–68] Netherlands [63] Germany [69] Denmark [70,71] Sweden [72–74] Norway [63]	Canada [75] Australia [76] Greece [64] Netherlands [77] Belgium [49,78] Germany [69] Sweden [79,80] Norway [81]
In vitro data (endpoints ranked according to potency. The study used for RCR calculation in bold)	**Antagonism of TR LOEC 100 nM** [28] Competitive binding to TTR IC_{50} 130 nM [29] T4 reduction and changed gene expression in zebrafish embryos LOEC 200 nM [82], LOEC 200 nM [83], LOEC 400 nM [84] Binding to TRα-LBD IC_{50} 16000 nM [85] Inhibition of iodide uptake in human sodium/iodide symporter (hNIS) assay LOEC 17000 nM [86]	**Metabolite activated CAR EC_{50} 900 nM** [30], EC_{50} 9800 nM [40] Inhibition of sulfotransferase IC_{50} 1410 nM [87] Decreased sodium/iodide symporter (NIS) in FRTL-5 cells LOEC 10000 nM [88] Reduced activity of iodotyrosine deiodinase LOEC 60000 nM [89] thyroid peroxidase (TPO) inhibition LOEC 253,000 nM [90]	**TTR binding IC_{50} 31 nM** [31], IC_{50} 3070 nM [91] Gene expression in zebra fish embryos LOEC 184 nM [92], LOEC 202 nM [93] Gene expression in zebra fish liver cells LOEC 400 nM [94] Growth hormone production in GH3 rat pituitary cells LOEC 1000 nM [95,96] Inhibition of rat disulfide isomerase IC_{50} 1180 nM [97] TR antagonism and TR-related effects LOEC 1000 nM [98], LOEC 3000 nM [99], IC_{50} 4600 nM [100], LOEC 10000 nM [101], IC_{50} 29500 nM [102] TRα transcriptional regulation IC_{75} 24000 nM [103] T-screen LOEC 10000 nM [104] Translocation of TRβ LOEC 25000 nM [105] Cell cycle regulation in human thyroid cells LOEC 75000 nM [106]	**TH reduction, gene and protein expression in zebra fish embryos LOEC 83 nM** [32], LOEC 83 nM [33] LOEC 104 nM [34] PXR activation LOEC 100000 nM [107]	**Effects on TH-inducible hepatic protein and TTR in chicken embryonic hepatocytes LOEC 1000 nM** [35] *Ex vivo Xenopus laevis* tadpole tail tip length regression LOEC 1000 nM [108] Increased TR-mediated gene expression LOEC 3120 nM [109] TTR binding IC_{50} 12000 nM [31] T-screen LOEC 21000 nM [31]
In vivo animal data (endpoints)	Reduced T4 levels in monkeys at estimated blood concentrations of 26,000 nM (NOAEL) and 76,000 nM (LOAEL) [110,111]	Reduced T4 levels in rats at estimated blood concentrations of 21 nM (NOAEL) and 214 nM (LOAEL) [112,113][a]	Reduced thyroid hormone levels in rats at blood levels estimated to 919 nM (NOAEL) [114,115]	Reduced T4 in male rodents at blood levels estimated to 4800 nM (BMDL) [116–118]	Reduced T4 in female rats with a NOAEL of 200 µg/g lipid in liver [119]. According to Szabo et al. [120] HBCD blood levels are approximately 33% of the hepatic adipose tissue levels after 10 days of exposure in mice. Based on this the blood level was estimated to 395 nM (NOAEL)

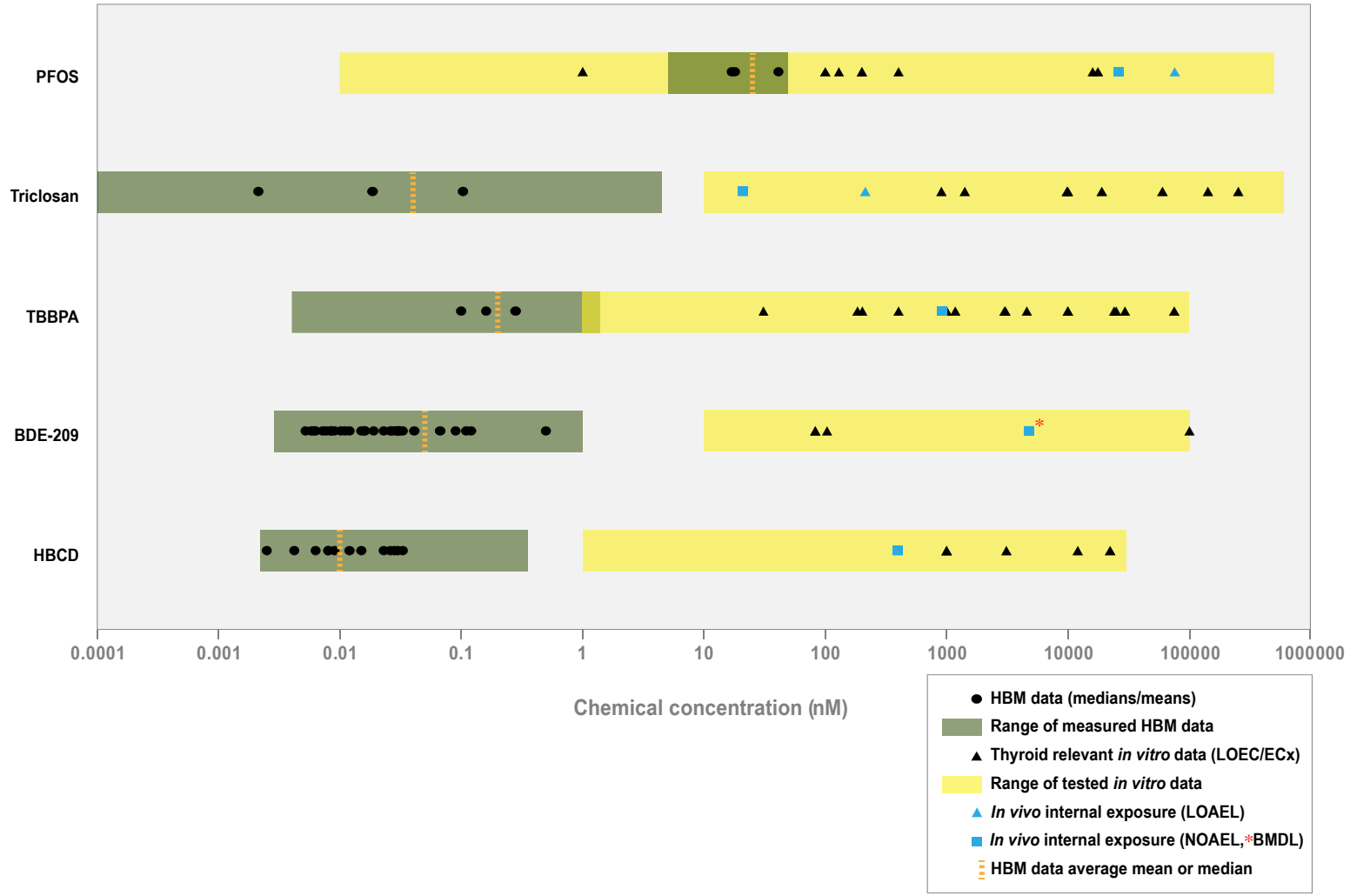


Figure 1: Overview of HBM data (green bars) and in vitro data (yellow bars) for PFOS and four other thyroid toxicants

5.2 Results

Ranking of the five compounds in terms of calculated RCR values based on in vitro NOEC values showed that PFOS was associated with the highest risk (RCR = 8) and HBCD with the lowest (RCR=0.0001):

PFOS >> TBBPA > BDE-209 > Triclosan >

Based on this analysis, exposure to PFOS alone is highlighted as a potential human health risk. The association is of even greater concern considering that humans are exposed to several perfluorinated compounds that may have the same or similar mode(s) of action, which can cause cumulative effects. The RCR ranking shows that PFOS needs further attention, although its use is restricted within the US and EU.

It should be noted, however, that the RCR values are subject to great uncertainties, both for the hazard and exposure data. The RCR values presented here should therefore be regarded as indicative values suggested as examples in this proposed framework, and not as values that should readily be used for risk assessment purposes.

As seen from the figure, the in vitro active concentrations and the blood levels at LOAELs and/or NOAELs from animal experiments were not that different. However, for PFOS effects are seen at lower concentrations in vitro than in vivo, whereas for triclosan the situation is opposite. This reflects that toxicity of some PFAS generally seem to be underestimated by animal studies and that human data and PBPK modelling are needed for a proper risk assessment of PFAS.

5.3 Conclusion

We used a case study on five thyroid toxic compounds with differential mechanistic profiles to investigate the potential use of HBM together with in vitro data for informing human risk assessment. We conclude that calculation of RCRs based on HBM and in vitro data is a helpful tool for risk ranking chemicals and for designing follow-up studies. Moreover, this approach may be used for pinpointing chemicals for which species differences may play a major role, thereby stressing the importance of basing the risk assessment on human-relevant data. PFOS turned out to have the highest RCR that exceeded 1, which may reflect that parts of the human population is exposed to levels that are of concern. With more human-relevant hazard data for PFAS, an approach like this may be useful for future risk assessments for individual compounds as well as for mixtures of PFAS. Our vision is that an in vitro/HBM approach can use data obtained in the HBM4EU project - in parallel to the NHANES project in the US - together with more comprehensive human relevant in vitro data to make ‘alternative’ risk assessment much more valuable to finally be able to ‘stand-alone’.

6 Discussion and conclusions

One of the goals of HBM4EU is to answer the policy questions related to the priority substances. The policy questions on PFAS are listed in the table below. Questions in bold are relevant for our task in 5.3.

Table 10: Policy questions on PFAS from the Scoping document

	Policy Questions PQ (Scoping Document on PFAS)
1	**What is the current exposure of the EU population to PFASs and do they exceed Guidance values (reference and HBM values), where available?**
2	Are there differences in exposure of the EU population to regulated and non-regulated PFASs?
3	**Has restriction of PFOS according to the POP Regulation led to a reduction in exposure, especially for children?**
4	Is exposure driven by diet, consumer exposure, occupation or environmental contamination?
5	Which areas and environmental media in Europe are contaminated with PFASs?
6	How can this feed into an assessment of the TDI for PFOS and PFOA set by EFSA?
7	What is the impact of a pending 2016 ECHA decision to restrict the manufacturing, marketing and use of PFOA under REACH?
8	Is it important to eliminate legacy PFASs from material cycles (i.e. waste electronic equipment) when implementing a circular economy in order to protect human health?
9	Can differences in PFASs profiles be observed in different population groups and time periods?
10	**What are the PFASs levels and health effects in vulnerable population groups?**
11	**How can mixture effects of environmental and human PFASs mixtures present to date be estimated?**
12	How can PFAS substances of relevance for human exposure and health be identified having in mind that more than 3000 substances are at the market?
13	How can identification and assessment (including data on (potential) adverse effects on human health and the environment) of alternatives currently hampered by CBI (Confidential Business Information) be facilitated?
14	How much are HBM values dependent on host characteristics and does this have implications for identifying vulnerable groups?

The relevant policy question for PFAS within Task 5.3 are:

1) What is the current exposure of the EU population to PFASs and do they exceed Guidance values (reference and HBM values), where available?
2) What are the PFASs levels and health effects in vulnerable population groups?
3) How can mixture effects of environmental and human PFASs mixtures present to date be estimated?

Within 5.3 we applied different approaches to address these policy questions based on the available risk assessments and selected human biomonitoring data. The CONTAM-Panel of EFSA stated in their scientific opinion that a considerable proportion of the population exceeds the established pTWIs for PFOS and PFOA and that the exceedances of the pTWIs for PFOS and PFOA at LB exposure estimates are of concern.

The aim of the WP5.3 PFAS team was to explore further possibilities to assess PFAS mixtures:

In chapter 3 mixtures risk assessment using the HI approach is presented. A detailed explanation is provided about the assumptions underlying the HI approach as such (e.g. common mode of action, linear and parallel dose-response curve) and the uncertainty around the knowledge about the validity around those assumptions. Also, more specific uncertainties are presented like using the Relative Potency Factors (RPF) approach as developed by RIVM and based on external exposure while HBM-based mixtures risk assessment aims to use internal HBM measurement data.

In a few HI calculations using a pseudonymised HBM data set, EFSAs pTWIs for PFOA and/or PFOS based on human data were used (Scientific Opinion on PFOA and PFOS by EFSA, 2018).

In order to demonstrate the challenges and obstacles of PFAS mixture risk assessment also HI calculations based on the recent ATSDR toxicological profile, which used animal data to establish provisional MRL values for intermediate exposure, as well as German HBMI values were taken forward as examples for a combined assessment. In addition, a mixture approach used for risk assessment to multiple PFAS in the Swedish population based on rodent hazard data from a publication by Borg et al., 2013 was applied.

Overall, our results indicate that if the mixture risk assessment is based on the RPF approach in conjunction with human data, the HI exceeds 1, which may signal that we have a problem that should be addressed. The driver of the mixture effect seems to be PFNA, which is likely from toxicokinetic differences based on external dose descriptors used in the RPF approach. It is likely that PFNA in reality has a potency more comparable to the other PFAS and therefore the HI may be overestimated. In contrast, we have included four major PFAS only in the mixture risk assessment (PFOA, PFOS, PFNA, PFHxS) and this may lead to an underestimation of the mixture effect evaluation, as humans are exposed to more PFAS. If we base our mixture risk assessment on animal data only – also those including internal exposure values – then the HI is well below 1 in the presented example. This result is also encumbered with great uncertainty due to the well-known species differences for PFAS. As we have taken into account all exposure routes by using aggregated HBM data, we would not expect other sources of the chemicals to be a great problem, although it is an underestimation that not all PFAS to which humans are exposed are included in the calculation.

Overall, our work on PFAS has brought forward very clearly some existing gaps in the knowledge and tools we have to perform HBM-based risk assessment.

The mixture methodology can be further developed, including tools for grouping of chemicals. However, there are currently pragmatic tools for mixture risk assessment to be used by risk

assessors (Boberg et al. 2019). The real bottleneck for implementing mixture risk assessment as we see it, is lack of hazard and exposure data for individual compounds (> 4000 PFAS in current use) and individuals. Here HBM4EU can contribute by delivering more HBM data to be used in future mixture risk assessment.

It is concluded that HBM-based information on internal exposure levels for PFAS has been and will be of great importance in risk assessments of PFAS (as has been demonstrated by EFSA for PFOA and PFOS), including also mixture risk assessments. The real bottleneck for performing risk assessments with lower uncertainties is the lack of human-relevant hazard data for many PFAS as rodent do not seem to reflect what is observed in humans (cholesterol effects, impaired vaccination response etc). This is probably due to differences in the PPARα response in rodents and in humans. Thus, we need more hazard data from human-relevant in vitro, ex vivo, and in silico studies in the future.

The approaches developed in this report rely on in vitro, in vivo and QSAR approaches to develop relevant estimates of hazard and relative potency in humans for variety of PFAS compounds. There is limited epidemiological evidence that allows a direct estimate of relative potency in humans. The different HBGVs derived for PFOA and PFOS, based on epidemiological data, point to differences in potency, for those two compounds at least. Populations normally have mixtures of multiple PFAS detectable in blood samples and the composition of such a mixture varies over time and strongly depends on the main sources of exposure (e.g. contaminated drinking water, diet and occupation). There are a number of different approaches to analysing these data – treating these serum concentrations as independent, treating them as mutual confounders by including them in regression models or seeking to identify some optimal combined exposure index. More work is needed to identify from these approaches the best way to characterise potency, based on epidemiological data, for the limited number of compounds with data in addition to PFOA and PFOS.

Overall, taking together the various suspected adverse effects in humans (ATSDR, 2018, EFSA, 2018 RIVM, 2017) and the widespread environmental and human exposure, a cautionary approach should be followed by risk managers.

6.1 Recommendations for the regulatory mixture risk assessment

The analysis we have performed in this report illustrates that HBM data have been used extensively to perform a detailed risk assessment of PFOA and PFOS and the value of using HBM data to inform risk assessment has clearly been demonstrated for these compounds. HBM data for other perfluorinated compounds are increasingly generated. However, as the fluorinated chemicals constitute a very diverse chemical class of more than 4,000 chemicals, there is a great need to extend HBM programs to include also many other types of PFAS. These include, but are not limited to, the precursors of the perfluorinated chemicals such as the polyfluorinated compounds (PAPS molecules), ethers that are replacing PFOS and PFOA (e.g. GenX) and many others. We therefore recommend to monitor the population in the future for the presence of not only perfluorinated chemicals but also other types of PFAS that are increasingly used. This is important from a health perspective but is also important in order to identify the ‘hot spot’ areas that seem to exist in Europe. The exposure assessment of PFOA and PFOS performed by EFSA identified various food items that were contaminated with these compounds, and this contamination varied a lot within the same category of food items. This indicates that some ‘hot spot’ areas in the outer environment seem to exist that are the source of the food contamination. Identification of these ‘hot spot’ areas is essential.

The drawback of using HBM data in risk assessments is that evaluation of exposure based on such data will always be a retrospective rather than a preventive approach. Furthermore, HBM data does usually not contribute with information concerning timing and source of the exposure, which is central to chemical regulation. However, if detailed contextual information is also collected or if human samples are collected in 'hot spot' areas, important information on sources may be deduced.

Reliance of regulatory authorities on experimental toxicity testing and limited number of PFAS studied in epidemiology has shown that traditional risk assessment may be inappropriate to assess human health risk. This has further been complicated by the fact that crucial documentation from companies has not always been made publicly available (Grandjean, 2018). Generally, more human relevant data are needed for many PFAS that together with HBM data can inform risk assessment both of PFAS' individually and in combination. Furthermore, development of better in silico tools including QSAR modelling and read across tools to inform risk assessment of PFAS is encouraged.

We have performed some initial mixture risk assessment of the four PFAS that according to our current knowledge constitute the major PFAS in human blood. We could perform this evaluation based on available animal hazard and exposure data, however the outcome of this assessment is highly uncertain due to important species differences. We then tried to base our calculations on human data, but realized that we were lacking human relevant hazard data for PFAS except for PFOA and PFOS. As a surrogate for internal RPFs, we used in our mixture calculations RPFs based on external data that is PODs based on animal data. We recommend to focus on development of RPFs based on internal data preferably based on human data if possible.

Our conclusion is that pragmatic tools are available for calculation of mixture effects of PFAS and other chemicals. However, the major bottleneck for implementing MRA is lack of relevant hazard and exposure data on single compounds and lack of knowledge on exactly how to group the chemicals in a MRA.

6.2 Future prospects

EFSA is likely to publish by the end of this year, next to PFOA and PFOS, a second scientific opinion on another subset of PFAS compounds including PFNA and PFHxS. It is foreseen also to address combined exposure to multiple PFAS. It is deemed justified to wait for the findings and conclusions of EFSA's CONTAM panel.

Some PFAS are subject to extensive assessments and risk reduction approaches at OECD and national level (OECD portal, 2018). Legislators in the US House of Representatives have recently launched a task force focused on the management of PFAS (ChemNews, 2019). Some PFAS are also subject to risk management option analyses, risk assessments and management measures and furthermore is topic of a specific working group at the ECHA.

Recently, a group of more than 50 international scientists and regulators prepared an action plan for the assessment and management of PFASs in the coming years as the risks of this compound group, which includes more than 4700 substances so far and which is characterized by extreme persistency remain a cause of emerging global concern (Ritscher et al. 2017).

Further work in cooperation with WP8 (HBM data on more PFAS) and WP15 (mixtures risk assessment) will enable further work towards HBM-based risk assessment of PFAS.

Within the HBM4EU WP8 aligned studies, it is planned to measure 12 PFAS. This will allow a refinement of exposure data and will provide for the first time harmonised and internal individual exposure data across Europe for the age group of teenagers of 12 to 19 years from 9 European

countries: NO, SE, SK, SI, GR, ES, FR, BE, DE. This will allow also a refinement of the risk assessment and applying a mixture assessment using scientifically further elaborated methods (planned EFSA assessment, work within HBM4EU WP 5, 13 and 15). It is planned to measure PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFBS, PFHxS, PFHpS, PFOS (sum of all isomers). However, it has to be noted that some parameters are already legacy PFAS. It can be assumed that concentrations of other PFAS compounds currently used as alternatives will increase. For regulatory measures, - e.g. preparing dossiers for identification of substances of very high concern or for a restriction proposal within REACH, it is important to prove that exposure occurs and extremely useful to report also HBM data. Therefore it is recommended to measure the compounds which are currently used as alternatives to the legacy PFAS, e.g. short chain PFAS which are mobile and contaminate groundwater and edible plants and also other relevant compounds such as GenX and Adona.

New targeted studies identifying a multitude of PFASs in human blood and urine including newly developed methods such as TOF or oxidisable fractions should be planned and performed, in order to be able to quantify also the so far unidentified compounds. These methods should be validated and harmonised.

It is also an urgent need to strengthen the efforts to characterise the toxicity of alternatives to legacy PFAS, especially concerning the immune system. More human relevant studies and data are needed for PFAS, because of the drawback of using rodent data for risk assessment. To gain more insight into mechanistic information HBM4EU WP 13 contributes to adverse outcome pathways development for human health endpoints identified in a variety of epidemiological studies.

Lastly, further work to trace and remove obstacles for an improved cooperation between the experts of epidemiology and toxicology is warranted. A more intensive cooperation on PFAS seems warranted in order to not only notice statistical correlations, but to be able to better tackle the issue of causal inference as well. Ways need to be found, how their data sets, assessment and interpretations can complement each other in order to foster scientific evidence building with respect to risks that go with exposure to a multitude of individual PFAS. These needs outreach far beyond only PFAS and should be taken up within HBM4EU and beyond in a more general way, for single substances exposures as well as mixed exposures. An overarching task force ‘bridging epidemiology and toxicology’ might be a way forward.

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Annex G: phthalates risk assessment (part A: general population)

D5.5 Substance-group specific risk assessment – General population risk assessment as part of the restriction dossier of the phthalates DEHP, BBP, DBP & DIBP under the European REACH legislation.

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1 Introduction

Phthalates are used as plasticizers in a wide variety of products. For the phthalates DEHP, BBP, DBP and DIBP, all classified as toxic to reproduction in category 1B, one was concerned that their use in Europe was not adequately controlled. Therefore, a restriction proposal was submitted by the Danish Environmental Protection Agency (D-EPA) in 2011 under the REACH Regulation (Registration, Evaluation, Authorisation, and Restriction of Chemicals; Regulation No 1907/2006), to restrict the placing on the market of articles containing these four phthalates (such as flooring, mattresses, footwear and office supplies). RAC (Risk Assessment Committee) and SEAC (Socio-economic Analysis Committee) adopted opinions not supporting the restriction. In 2016, D-EPA and the European Chemicals Agency (ECHA) prepared a new restriction dossier for the four phthalates, which built further on the previous dossier, presenting among others additional information and assessment covering the hazard and new information on exposure (especially DEMOCOPHES biomonitoring data). One year later, the opinions of RAC and SEAC on the restriction dossier were adopted (ECHA, 2017b).

This document starts with a summary of the use of human biomonitoring (HBM) data in the restriction. This document further aims to provide insight in how new HBM data could improve the chemical risk assessment of the phthalates DEHP, BBP, DBP, and DIBP, which served as the backbone of the restriction, and whether the impact of the restriction can be monitored by making use of HBM data. This is in line with the objectives of WP5.3, in which is aimed to investigate how chemical risk assessment can be improved through the use of HBM data and to bring the results of HBM forward in terms of possible options for policy action and risk management. Specific questions to be answered in WP5.3 for the phthalates chemical group are:

- How was HBM data used in the restriction and what were the strengths and limitations?
- Would it be possible to improve the risk assessment based on new data or new studies?
- Can HBM be used to monitor the effect of the restriction, both on the restricted phthalates and possible alternatives? And what would be a viable approach?

2 Methodology

The restriction proposal and the opinions of RAC and SEAC form the basis of this document. Consequently, the first part of this document, in which the role of HBM data within the restriction is discussed, will mainly focus on the four phthalates subject to restriction: Bis(2-ethylhexyl) phthalate (DEHP), Benzyl butyl phthalate (BBP), Dibutyl phthalate (DBP), and Diisobutyl phthalate (DiBP). The second part, which discusses the impact of the restriction and the role HBM data could have in quantifying the effects, will also take into consideration information on the substitute phthalates Diethyl phthalate (DEP), Dimethyl phthalate (DMP), Diisononyl phthalate (DiNP) and Diisodecyl phthalate (DiDP), Di(2-propylheptyl) phthalate (DPHP), and the alternative plasticizer Diisononyl cyclohexane-1,2-dicarboxylate (DINCH).

This document will follow the outline as presented below:

Chapter 3: Summary of the hazard characterization as established in the restriction proposal (D-EPA, 2016) and the RAC and SEAC opinions (ECHA, 2017b).

Chapter 4: Summary of the exposure assessment as established in the restriction proposal (D-EPA, 2016) and the RAC and SEAC opinions (ECHA, 2017b), with specific focus on the use of HBM data in the exposure assessment.

Chapter 5: Risk characterization and uncertainty analysis as discussed in the RAC and SEAC opinions (D-EPA) and (ECHA), with the main focus on the strengths and limitations of using HBM data in the restriction.

Chapter 6: Improving the risk assessment by making use of new HBM data

- Evaluation of additional HBM data, among others for sensitive subgroups
- Using the health based guidance values for DINCH and DEHP
- Time trends of the restricted phthalates DEHP, BBP, DBP, and DIBP, the substitute phthalates DEP, DMP, DiNP and DiDP/DPHP, and the alternative plasticizer DINCH

Chapter 7: Conclusion

3 Summary of hazard characterisation

Between 2001 and 2009, DEHP, BBP, DBP and DIBP were subject to harmonized classification, and all were regarded toxic to reproduction in category 1B according to CLP Regulation (EC) No. 1272/2008. In the restriction proposal, these chemicals were presented as a group of chemicals with similar physicochemical properties, sharing the same anti-androgenic mode of action, and having similar use. The scope of the restriction was limited to these four phthalates, because they were all identified as SVHC (Substance of Very High Concern). Other phthalates, however, which may contribute to combined risk, were not taken into account.

In the male rat, phthalates are known to cause a spectrum of effects via an anti-androgenic mode of action, also referred to as the phthalate syndrome. Specific effects are inhibition of foetal testosterone production, reduction of male anogenital distance, decreased gene expression related to steroid biosynthesis, increased permanent nipple retention in male offspring, increased incidence of genital malformations (hypospadias and cryptorchidism), delayed puberty onset, reduced semen quality and testicular changes (including decreased testes and epididymides weight), tubular atrophy and increased incidence of Leydig cell hyperplasia. The latter effects are also considered relevant for male humans (ECHA, 2017b).

Dose-response assessment

Derived no effect levels (DNELs) were calculated for the four phthalates, using No Observed Adverse Effect Levels (NOAELs) for anti-androgenic effects observed in developmental animal experiments as point of departure (PoD) (**Table 1**). These DNELs were consistent with those previously agreed upon by RAC, except from the DNEL for DIBP, which was newly derived based on read-across from DBP. DNELs established for the external oral dose were transformed into internal doses, assuming an oral absorption fraction for DEHP in rats of 0.7 and of 1 for the other three compounds.

Table 1: DNEL derivation for DEHP, BBP, DBP, and DIBP (ECHA, 2017b).

Chemical	Adverse effect(s) observed	PoD	AFs[a]	DNEL external oral dose (mg/kg bw/day)	DNEL internal dose (mg/kg bw/day) [b]	Source
DEHP	Testicular effects (germ cell depletion, reduced testis weight) in male offspring	NOAEL of 4.8 mg/kg bw/day	4*2.5*10 = 100	0.048	0.034	(Wolfe and Layton, 2003)
DBP	Delayed germ cell development and degeneration and atrophy of mammary gland alveoli	LOAEL of 2 mg/kg bw/day	4*2.5*10*3 = 300	0.0067	0.0067	(Lee *et al.*, 2004)
DIBP	Based on read-across from DBP, taking into account 25% less potency of DIBP compared to DBP (as observed in (Saillenfait *et al.*, 2008)	LOAEL of 2.5 mg/kg bw/day	4*2.5*10*3 = 300	0.0083	0.0083	(Lee *et al.*, 2004)
BBP	Reduced anogenital distance in male offspring, reduced reproductive organ weights and altered sperm counts and motility	NOAEL of 50 mg/kg bw/day	4*2.5*10 = 100	0.50	0.50	(Nagao *et al.*, 2000; Tyl *et al.*, 2004; Aso *et al.*, 2005; Ahmad *et al.*, 2014)

[a]Assessment Factors. Default factors of 4 for rat allometric scaling, 2.5 for remaining interspecies differences, 10 for intraspecies differences, and 3 for LOAEL to NAEL extrapolation for the substances without NOAEL.
[b]Assuming an oral absorption fraction for DEHP in rats of 0.7 and 1 for the other three compounds

4 Summary exposure assessment

The exposure assessment was based especially on urinary biomonitoring data from the EU-wide DEMOCOPHES project, given it was recent, consisted of a large sample size, and was representative for the European population (Den Hond *et al.*, 2014). The use of this data was unique, as exposure estimates are usually derived from exposure modelling due to the lack of human biomonitoring data available, harmonized on EU level. For the DEMOCOPHES project, morning urine spot samples were collected from mother-child pairs in 17 European countries during the period 2011-12. The median age of the mothers was 39, and the age of the children varied from 6 to 11.

Data were transformed from the creatinine corrected urinary metabolite concentrations (in µg/L) to internal intake estimates (in µg/kg bw/day) using the formula from (Frederiksen *et al.*, 2013):

Equation 1:

$$DI\ \left(\frac{\mu g}{kg * day}\right) = \frac{\left[\left(\frac{UE_{m1crea}\left(\frac{\mu g}{g_{crea}}\right)}{MW_{m1}\left(\frac{\mu g}{\mu mol}\right)}\right)+\left(\frac{UE_{m2crea}\left(\frac{\mu g}{g_{crea}}\right)}{MW_{m2}\left(\frac{\mu g}{\mu mol}\right)}\right)+\cdots\right] * MW_p\left(\frac{\mu g}{\mu mol}\right) * CE_{smoothed}\left(\frac{g}{day}\right)}{FUE * BW(kg)}$$

Where

DI = estimation of daily intake of phthalate diesters;
$CE_{smoothed}$ = 24-h urinary creatinine excretion;
BW = body weight ;
$UE_{m1\ crea}$, $UE_{m2\ crea}$,… = creatinine adjusted urinary concentration of phthalates metabolites;
MW_{m1}, MW_{m2} ,... = molecular masses of each of the respective metabolites;
MW_p = molecular mass of the specific phthalate diester; and
FUE = fraction of the phthalate diester excreted in urine.

As data from the DEMOCOPHES project was largely unpublished in 2016, detailed information of the participants, such as body weight, age, height, creatinine excretion levels, or urinary metabolite levels was lacking. This led to some loss of precision, as the estimated daily intake was calculated from the creatinine corrected urinary concentration of metabolites using default values (i.e. a fixed value for the 24h creatinine excretion and a country-specific median for body weight) (D-EPA, 2016). This resulted in the median and 95th percentile intake estimates for mother and child per country (Figure 1).

In addition, RAC took into account other biomonitoring studies as well (ECHA, 2017b). A study performed among 239 mother-child pairs collected between 2007 and 2008 in Greece (Myridakis *et al.*, 2015) was added to the DEMOCOPHES data, thereby increasing the number of included countries to 18 in total (Table 2). However, as the age group of the children was younger (2-3), only the data of the mothers was included and combined with the DEMOCOPHES data (Table 3).

				intake			
				DEHP	DBP	BBP	DiBP
Country	N	Population		µg/kg/d	µg/kg/d	µg/kg/d	µg/kg/d
BE	125	Mother	P50	1.49	0.84	0.18	1.04
			P95	4.92	2.64	0.65	5.02
	125	Child	P50	2.11	0.98	0.23	1.43
			P95	12.06	2.90	0.92	8.60
CH	117	Mother	P50	1.15	0.46	0.10	0.50
			P95	5.83	1.82	0.43	1.61
	119	Child	P50	2.11	0.64	0.12	0.64
			P95	7.45	1.91	0.81	2.08
CY	59	Mother	P50	1.03	0.46	0.06	1.51
			P95	14.99	1.33	0.30	3.62
	60	Child	P50	1.42	0.57	0.09	1.54
			P95	7.77	1.51	0.41	3.60
CZ	117	Mother	P50	2.53	1.83	0.13	NA
			P95	8.05	4.98	1.30	NA
	120	Child	P50	4.41	3.10	0.19	NA
			P95	14.03	8.90	1.49	NA
DE	116	Mother	P50	1.39	0.86	0.12	0.68
			P95	3.82	2.28	0.54	1.89
	120	Child	P50	2.45	1.19	0.15	1.09
			P95	7.26	3.66	1.01	3.06
DK	143	Mother	P50	1.61	0.66	0.13	1.22
			P95	5.37	1.28	0.52	3.30
	142	Child	P50	2.84	0.93	0.21	1.73
			P95	7.75	2.03	1.00	4.92
ES	118	Mother	P50	3.17	1.00	0.24	1.25
			P95	8.70	2.25	0.96	2.67
	119	Child	P50	4.74	1.30	0.37	1.62
			P95	12.05	6.03	1.39	7.07
HU	115	Mother	P50	2.21	1.03	0.11	0.00
			P95	8.49	3.21	0.53	0.00
	117	Child	P50	3.47	1.49	0.17	0.00
			P95	12.86	4.57	0.78	0.00
IE	120	Mother	P50	2.05	0.56	0.08	0.71
			P95	6.58	1.58	0.54	3.00
	120	Child	P50	3.32	0.68	0.12	1.09
			P95	10.27	1.75	0.57	3.91

				intake			
				DEHP	DBP	BBP	DiBP
Country	N	Population		µg/kg/d	µg/kg/d	µg/kg/d	µg/kg/d
LU	58	Mother	P50	1.08	0.60	0.10	0.65
			P95	4.98	1.42	0.41	2.29
	60	Child	P50	1.63	0.77	0.12	1.09
			P95	3.84	1.69	0.58	5.98
PL	119	Mother	P50	2.89	1.37	0.11	1.51
			P95	12.39	5.59	0.71	5.94
	115	Child	P50	4.57	2.14	0.24	2.93
			P95	17.31	7.58	1.63	10.07
PT	117	Mother	P50	2.47	0.65	0.15	0.86
			P95	11.59	1.51	0.47	2.52
	116	Child	P50	2.82	0.81	0.20	1.05
			P95	8.91	2.25	1.05	3.41
RO	117	Mother	P50	3.13	0.72	0.07	1.01
			P95	34.60	1.70	0.32	2.79
	119	Child	P50	4.23	1.11	0.10	1.41
			P95	29.85	3.97	0.54	5.10
SE	96	Mother	P50	1.73	1.79	0.34	NA
			P95	5.84	4.96	2.25	NA
	97	Child	P50	3.21	2.27	0.60	NA
			P95	11.16	6.46	2.60	NA
SI	120	Mother	P50	NA	0.56	0.12	NA
			P95	NA	2.71	0.50	NA
	120	Child	P50	NA	0.84	0.16	NA
			P95	NA	2.70	0.75	NA
SK	125	Mother	P50	2.53	1.87	0.11	NA
			P95	7.11	5.32	0.44	NA
	127	Child	P50	4.90	2.70	0.18	NA
			P95	14.10	7.46	0.90	NA
UK	21	Mother	P50	1.00	0.42	0.06	0.47
			P95	2.69	0.95	0.14	2.20
	21	Child	P50	2.53	0.73	0.11	0.77
			P95	5.41	1.94	0.62	2.33

Figure 1: Median and 95th percentile intake estimates for mother and child per EU country presented in µg/kg bw/day (ECHA, 2017a).

Table 2: Overall intake estimates for a representative sample of the European population based on published DEMOCOPHES data (Den Hond *et al.*, 2014), data from Greece (Myridakis *et al.*, 2015), and these data combined for mothers (ECHA, 2017b).

Intake (µg/kg bw/day)								
	N	Median	P95	Maximum	N	Median	P95	Maximum
Children EU DEMOCOPHES					**Children (Myridakis *et al.*, 2015)**			
DEHP	1816	3.3	12	256	239	4.0	21.6	69.6
DBP	1355	1.0	4	25	239	1.0	6.6	50.8
BBP	1816	0.2	1.2	17	239	0.2	1.3	9
DIBP	1355	1.4	5.0	49	239	1.4	8.2	36
Mothers EU DEMOCOPHES					**Mothers (Myridakis *et al.*, 2015)**			
DEHP	1800	2.1	8.3	123	239	4.4	25.6	1015
DBP	1347	0.7	2.1	65	239	1.9	11.4	4840
BBP	1800	0.1	0.7	14	239	0.3	1.8	9.9
DIBP	1347	10.9	3.2	12	239	2.1	11.0	30.6

Table 3: Overall intake estimates for a representative sample of the European population (mothers) based on combined published DEMOCOPHES data (Den Hond *et al.*, 2014) and data from Greece (Myridakis *et al.*, 2015) (ECHA, 2017b).

Intake (µg/kg bw/day)				
Mothers EU DEMOCOPHES and (Myridakis *et al.*, 2015)*				
	N	**Median**	**P95**	**Maximum**
DEHP	2039	2.37	10.33	1015
DBP	1586	0.88	3.50	4840
BBP	2039	0.12	0.83	14
DIBP	1586	1.08	4.38	30.6

*Combined by weighted averaging, only for the mothers as the children were different of age.

Furthermore, data from Fromme *et al.* (2013) was provided for illustrative purposes, as this study investigated a relatively young age group (infants between 15-21 months old). Due to the small sample size, this study was not used for the calculation of exposure estimates. Still, this study suggested that exposure to infants was probably higher than exposure to children between the age of 6-11, and therefore the human biomonitoring derived estimated daily intake for children in the risk assessment could potentially be underestimated (ECHA, 2017b).

Human biomonitoring data generally does not provide insight into the contribution of different sources to the overall exposure. Therefore, exposure modelling was used to fill the remaining data gaps. Exposure estimates were calculated for exposure via food, contact with consumer products, and the indoor environment per phthalate, for infants, children, and women by deterministic modelling. Furthermore, probabilistic modelling (Monte Carlo) was used to calculate the aggregated exposure per phthalate, per subpopulation. All exposure estimates were converted into internal dose estimates (µg/kg bw/day) by using dermal and oral absorption rates (dermal absorption fractions of 0.05 (DEHP and BBP) and 0.1 (DBP and DiBP); oral absorption fraction of 0.7 for DEHP and of 1 for the other three compounds). This resulted in an overview of the contribution to the aggregated exposure per phthalate, per exposure source, per age group in µg/kg bw/day (Table 4).

Table 4: Aggregated exposure from indoor environment, food and contact with articles for four phthalates established for infants, children, and women, presented as internal dose estimates in µg/kg bw/day (D-EPA, 2016).

	Infants			Children			Mothers		
	Typical	RWC	MC RWC	Typical	RWC	MC RWC	Typical	RWC	MC RWC
DEHP									
Indoor	4.22	21.85	21.85	0.93	5.51	5.51	0.48	2.52	2.52
Food	4.66	7.09	7.09	3.50	5.38	5.38	1.49	2.86	2.86
Articles	3.49	27.32	27.67	2.39	17.91	17.26	2.12	7.63	12.06
Total	12.37	56.26	56.61	6.82	28.80	28.15	4.09	13.01	17.45
Monte Carlo			42.98			22.38			14.17
DBP									
Indoor	0.28	1.47	1.47	0.04	0.27	0.27	0.02	0.12	0.12
Food	0.70	1.24	1.24	0.20	0.30	0.30	0.08	0.16	0.16
Articles	1.20	9.22	6.48	0.83	6.22	4.39	0.74	2.65	3.17
Total	2.18	11.93	9.19	1.07	6.79	4.96	0.84	2.92	3.45
Monte Carlo			6.63			4.63			3.27
DIBP									
Indoor	0.27	1.41	1.41	0.04	0.25	0.25	0.02	0.11	0.11
Food	1.03	9.02	9.02	0.42	0.64	0.64	0.14	0.28	0.28
Articles	1.06	8.16	6.74	0.73	5.50	4.49	0.65	2.34	3.09
Total	2.37	18.59	17.18	1.19	6.40	5.39	0.82	2.74	3.48
Monte Carlo			12.19			4.94			3.28
BBP									
Indoor	0.08	0.42	0.42	0.01	0.08	0.08	0.01	0.03	0.03
Food	0.00	0.00	0.00	0.12	0.21	0.21	0.05	0.12	0.12
Articles	0.31	2.43	1.75	0.21	1.59	1.13	0.19	0.68	0.77
Total	0.39	2.85	2.17	0.34	1.87	1.41	0.25	0.83	0.92
Monte Carlo			1.90			1.25			0.83

Typical = Typical case scenario
RWC = Reasonable worst case scenario
RWC MC = Monte Carlo simulation of the reasonable worst case scenario

5 Risk characterisation and uncertainty analysis

The risk characterization ratios (RCRs) established using the 95th percentile of DEMOCOPHES HBM data illustrated that children in 13 out of 17 countries were at risk, and women were at risk in 6 out of 17 countries (Figure 2). Additionally, comparison of RCRs based on probabilistic modelling with HBM-based RCRs illustrated that there was an overall good correspondence between the RCRs established, whereas exposure modelling appeared to underestimate risks slightly in countries with high exposure levels (Table 5) (ECHA, 2017b). Exposure modelling further suggests that infants may be higher exposed than children, which could not be confirmed with HBM data (see section 5.1, page 9). Furthermore, data presented in (Myridakis *et al.*, 2015) was used together with the DEMOCOPHES exposure estimates to establish an overall risk for the European population (Table 6). These data illustrated that both for children and women in the European population, risks were not adequately controlled. Modelling furthermore suggested that the aggregated exposure to the phthalates could be twice as high for infants compared to the exposure determined for children, and hence infants would be especially at risk since even short elevated exposure within the critical windows of exposure is sufficient to cause adverse health effects (ECHA, 2017b). The estimate of the risk of infants however could not be validated due to a lack of robust HBM data for this group.

	Mother						Child					
Country	N	DEHP	DBP	BBP	DIBP	SUM	N	DEHP	DBP	BBP	DIBP	SUM
SI	120	0.2	0.4	0.0	*NA*	0.6	120	0.2	0.4	0.0	*NA*	0.6
UK	21	0.1	0.1	0.0	0.3	0.5	21	0.2	0.3	0.0	0.3	0.7
CH	117	0.2	0.3	0.0	0.2	0.6	119	0.2	0.3	0.0	0.3	0.8
CY	59	0.4	0.2	0.0	0.4	1.1	60	0.2	0.2	0.0	0.4	0.9
PT	117	0.3	0.2	0.0	0.3	0.9	116	0.3	0.3	0.0	0.4	1.0
IE	120	0.2	0.2	0.0	0.4	0.8	120	0.3	0.3	0.0	0.5	1.0
HU	115	0.2	0.5	0.0	*NA*	0.7	117	0.4	0.7	0.0	*NA*	1.1
LU	60	0.1	0.2	0.0	0.3	0.6	60	0.1	0.3	0.0	0.7	1.1
DK	143	0.2	0.2	0.0	0.4	0.7	142	0.2	0.3	0.0	0.6	1.1
DE	116	0.1	0.3	0.0	0.2	0.7	120	0.2	0.5	0.0	0.4	1.1
SE	96	0.2	0.7	0.0	*NA*	0.9	97	0.3	1.0	0.0	*NA*	1.3
SK	125	0.2	0.8	0.0	*NA*	1.0	127	0.4	1.1	0.0	*NA*	1.5
CZ	117	0.2	0.7	0.0	*NA*	1.0	120	0.4	1.3	0.0	*NA*	1.7
BE	125	0.1	0.4	0.0	0.6	1.1	125	0.4	0.4	0.0	1.0	1.8
RO	117	1.0	0.3	0.0	0.3	1.6	119	0.9	0.6	0.0	0.6	2.1
ES	118	0.3	0.3	0.0	0.3	0.9	119	0.4	0.9	0.0	0.9	2.1
PL	119	0.4	0.8	0.0	0.7	1.9	115	0.5	1.1	0.0	1.2	2.9

Figure 2: Risk Characterization Ratios for the summed exposure to four phthalates as estimated from the 95th percentile HBM urine levels in the DEMOCOPHES project (2011-2012).

Table 5: Risk Characterization Ratios for exposure estimates (Monte Carlo simulation; reasonable worst case) and the range of 95th percentile of biomonitoring exposure estimates established for different European countries.

	Indoor	Food	Articles	Total	Aggregated RCR (MC)	95th percentile biomonitoring	Combined RCR (MC)
Infants							
DEHP	0.64	0.21	0.81	**1.67**	**1.26**	NA	**2.63**
DBP	0.22	0.19	0.97	**1.37**	**1.14**	NA	
DIBP	0.17	**1.09**	0.81	**2.07**	**1.47**	NA	
BBP	0.00	0.00	0.00	0.00	0.00	NA	
Total	**1.03**	**1.48**	**2.60**	**5.11**		NA	
Combined RCR per source (MC)	0.76	**1.34**	**1.69**				
Combined RCR (MC)	**2.69**						
Children							
DEHP	0.16	0.16	0.51	0.83	0.66	0.16-0.88	**1.34**
DBP	0.04	0.04	0.65	0.74	0.69	0.28-**1.21**	
DIBP	0.03	0.08	0.54	0.65	0.60	0.25-**1.21**	
BBP	0.00	0.00	0.00	0.00	0.00	0.00-0.01	
Total	0.23	0.28	**1.71**	**2.22**		0.75-**2.94**	
Combined RCR per source (MC)	0.18	0.25	**1.11**				
Combined RCR (MC)	**1.34**						
Mothers							
DEHP	0.07	0.08	0.35	0.51	0.42	0.08-**1.02**	**0.90**
DBP	0.02	0.08	0.47	0.51	0.49	0.15-0.89	
DIBP	0.01	0.02	0.37	0.42	0.40	0.27-0.72	
BBP	0.00	0.03	0.00	0.00	0.00	0.00-0.00	
Total	0.11	0.00	**1.20**	**1.45**		0.50-**1.98**	
Combined RCR per source (MC)	0.08	0.12	0.81				
Combined RCR (MC)	0.91						

RCRs > 1 are highlighted in bold; Typical = Typical case scenario; RWC = Reasonable worst case scenario ; RWC MC = Monte Carlo simulation of the reasonable worst case scenario

Table 6: Overall RCRs for a representative sample of the European population based on published DEMOCOPHES data (Den Hond *et al.*, 2014), data from Greece (Myridakis *et al.*, 2015), and these data combined for mothers (ECHA, 2017b) for the year 2011.

RCRs based on biomonitoring			
	N	**Median**	**P95**
Children EU DEMOCOPHES (6-11 y)			
DEHP	1816	0.1	0.4
DBP	1355	0.1	0.6
BBP	1816	0.0	0.0
DIBP	1355	0.2	0.6
Sum	-	0.4	**1.5**
Mothers EU DEMOCOPHES			
DEHP	1800	0.1	0.2
DBP	1347	0.1	0.3
BBP	1800	0.0	0.0
DIBP	1347	0.1	0.4
Sum	-	0.3	0.9
Children (~2 y) (Myridakis *et al.*, 2015)			
DEHP	239	0.12	0.64
DBP	239	0.15	0.99
BBP	239	0.00	0.00
DIBP	239	0.17	0.99
Sum	-	0.44	**2.61**
Mothers (Myridakis *et al.*, 2015)			
DEHP	239	0.13	0.75
DBP	239	0.28	**1.70**
BBP	239	0.00	0.00
DIBP	239	0.25	**1.33**
Sum	-	0.67	**3.78**
Mothers EU DEMOCOPHES and (Myridakis *et al.*, 2015)			
DEHP	2039	0.07	0.30
DBP	1586	0.13	0.52
BBP	2039	0.00	0.00
DIBP	1586	0.13	0.53
Sum	-	0.33	**1.36**

RCRs > 1 highlighted in bold

5.1 Uncertainty analysis

The main conclusion following from the uncertainty analysis performed in the restriction proposal and the RAC and SEAC opinions was that risks were rather underestimated than overestimated. It was indicated that lower DNELs could be derived for all phthalates, based on other studies or more sensitive end-points which are still under evaluation. Additionally, other chemicals with the same assumed mode of action could contribute to the mixture effect on male reproductive development (phthalates and non-phthalates), which consequently also may contribute to the total risk. Other uncertainties were associated with the use of human biomonitoring in the exposure assessment, which will briefly be discussed below.

First, there was uncertainty involved in the methods of the human biomonitoring studies in terms of harmonization. It was observed that the median and the P95 of the exposure estimates for both age groups in Myridakis *et al.* (2015) were considerably higher compared to those of the DEMOCOPHES project (Table 2, Section 4). One of the factors proposed to have impact on this difference is the methodology used to calculate the exposure estimates. Two extrapolation methods generally can be used to estimate the urinary metabolite levels: the volume-based method and the creatinine excretion-based method. In the DEMOCOPHES study, the creatinine excretion-based extrapolation method was used to estimate the daily intake. In Myridakis *et al.* (2015), volume reference values were used to estimate the daily intake for the four phthalates, which resulted in higher exposure estimates (ECHA, 2012a).

Second, there was uncertainty involved in the methods of the human biomonitoring studies in terms of sampling and data transformation. For the DEMOCOPHES project, urine spot samples were taken in the morning. As excretion of phthalate metabolites is dependent on the rate of metabolism, the moment of sample withdrawal may not have provided a representative image of the mean daily intake (ECHA, 2017b). Another factor that potentially influenced the exposure estimates was the transformation of the creatinine corrected urinary metabolite concentrations to internal intake estimates. Equation 1 was required for transformation of the data, and required certain default values such as the molar urinary excretion fraction of phthalate diester (FUE) and mean body weight per country. Uncertainty is involved with the molar urinary excretion fraction of phthalate diester, as this fraction is considered to be influenced by inter- and intra-individual variation in phthalate metabolism (ECHA, 2012a). Furthermore, this fraction has been derived from studies on adult humans, and no additional FUE for children has been established. Instead, the fraction for adults was also used for children, which assumes that there are no age related differences in metabolic rate. For this age group, the use of the adult fraction could have resulted in underestimation of the exposure to DBP, BBP, and DIBP (ECHA, 2017b).

Third, there was uncertainty involved in the selection of a sufficiently representative study population. For some EU countries only a small sample size was available (around 120 individuals), thereby increasing the risk that highly exposed individuals are not included in the sample. Despite the fact that the 95th percentile of the distribution was taken as a worst-case scenario, this value may therefore not be representative for highly exposed subpopulations. Infants are mentioned as a subgroup with potentially higher exposure, but also patients chronically exposed to phthalates via hemodialysis or through medicine intake (i.e. phthalates in the enteric coatings of capsules). Exposure modelling suggests that infants may be higher exposed than children, but this could not be confirmed with HBM data. Patients with hemodialysis were not admitted to the DEMOCOPHES study, thus for patients regularly undergoing treatment with DEHP containing medical devices the risks are likely underestimated in this risk assessment. However, adding RCRs based on P95 for the four phthalates may have led to some overestimation of the risk, albeit RAC noted high exposure to these four phthalates is not uncommon (ECHA, 2017b).

6 Improving the RA by biomonitoring

6.1 Evaluation of the available biomonitoring data

Because only four phthalates were included in the authorisation at the time the scope of the restriction was determined, no other phthalates have been evaluated in the restriction. However, other phthalates that were not taken into account, may contribute to combined risk. For this reason, the evaluation of the biomonitoring data was extended to all phthalates for which data is available.

Several recent publications are available which observed human exposure to phthalates by means of human biomonitoring of metabolites in urine samples, which were not taken into consideration by the time the restriction was written, or were not available yet (Frederiksen *et al.*, 2014; Schutze *et al.*, 2014; Schutze *et al.*, 2015; Gyllenhammar *et al.*, 2017; Koch *et al.*, 2017; Larsson *et al.*, 2017; Schoeters *et al.*, 2017). These studies focussed on DEHP, BBP, DnBP and DiBP (Frederiksen *et al.*, 2014; Gyllenhammar *et al.*, 2017; Koch *et al.*, 2017; Larsson *et al.*, 2017; Schoeters *et al.*, 2017), substitute phthalates DEP, DMP, DiNP, DiDP, and DPHP (Frederiksen *et al.*, 2014; Schutze *et al.*, 2015; Gyllenhammar *et al.*, 2017; Koch *et al.*, 2017; Larsson *et al.*, 2017), and the alternative plasticiser DINCH (Schutze *et al.*, 2014; Gyllenhammar *et al.*, 2017; Larsson *et al.*, 2017). Several different (sensitive) subgroups were studied, such as children, adolescents, young adults, and mothers from the European countries Austria, Belgium, Germany, Denmark, and Sweden. Frederiksen *et al.* (2014), (Hartmann *et al.*, 2015) and Larsson *et al.* (2017) did present exposure estimates at one time point. The other studies provided data able to observe trends over time.

In HBM4EU, the question was posed whether it would be of additional value to supplement the risk assessment in the restriction by new data in an attempt to improve the existing risk assessment. In the restriction it is noted that many studies were available during the time the document was under development, focussing on the chemicals subject to restriction. However, it was decided not to include these studies in the analysis, as several factors were inconsistent among them (such as the sample period, geographic location of residence of the study population, size of the study population, methodology to estimate the daily intake, and different age groups). From the restriction it may be concluded that exposure differs among European countries, and data from one country may not necessarily be of additional value to make a statement concerning the risk of phthalate exposure on a European level. However, several data gaps were noted in the existing data, such as a lack of data for infants and hemodialysis patients, groups that are especially at risk. As of today, no additional data has become available that studied these specific groups.

In WP5.2, HBM health based guidance values (HBGV) were derived for DEHP and DINCH. These values provide a reference concentration of a metabolite (or combined concentration of two metabolites) in urine below which no adverse health effects are expected. As with derivation of other reference values, they are based on NOAELs or Benchmark Doses (BMDs) determined using animal toxicity data, or other benchmark values derived from epidemiological data.

The main difference in the derivation of the HBM4EU HBGV and the DNEL used in the restriction is that the first compares the metabolite concentration in urine directly with the HBGV. In the restriction, the internal intake estimates of DEHP were calculated from urinary metabolite concentrations using Equation 1, and these were compared to a transformed internal DEHP DNEL concentration to estimate the risk. Ideally, no discrepancies in the estimated risk characterization ratios should result from these calculations. In section 6.2, the HBM4EU reference values of DEHP

and DINCH will be used to 1) expand on the existing risk assessment of DEHP and 2) estimate the potential risk from DINCH exposure in the general population based on recent HBM data.

In addition, section 6.3 will focus on studying the implementation and effectiveness of the restriction. Therefore, time trends of exposure to the restricted phthalates, alternative phthalates, and the alternative plasticizer DINCH will be studied. Ideally, one would see a decline in exposure to the restricted phthalates. Furthermore, it is expected to observe an increase in the exposure to alternative phthalates and DINCH.

6.2 Human biomonitoring guidance values

As part of WP 5.2, EU-wide health based guidance values were derived for DEHP and DINCH (for the rationale behind the derivation of these values see the WP 5.2 Deliverable Report of December 2017). For DEHP, a HBM I $HBGV_{GenPop\ (5\text{-}oxo\text{-}MEHP\ and\ 5OH\text{-}MEHP)}$ for adults of 500 µg/L and a HBM I $HBGV_{GenPop\ (5\text{-}oxo\text{-}MEHP\ and\ 5OH\text{-}MEHP)}$ for children of 340 µg/L were derived. Additionally, a HBM I $HBGV_{GenPop\ (5\text{-}cx\text{-}MEPP\ and\ 5OH\text{-}MEHP)}$ for adults of 570 µg/L and a HBM I $HBGV_{GenPop\ (5\text{-}cx\text{-}MEPP\ and\ 5OH\text{-}MEHP)}$ for children of 380 µg/L were established. For DINCH, a HBM I $HBGV_{GenPop\ (OH\text{-}MINCH\ and\ cx\text{-}MINCH)}$ for adults of 4.5 mg/L and a HBM I $HBGV_{GenPop\ (OH\text{-}MINCH\ and\ cx\text{-}MINCH)}$ for children of 3 mg/L were derived **Table 7**. These HBM I HBGVs for the general population are presented in µg/L. However, the data available for DEHP and DINCH are presented in their creatinine corrected form (i.e. µg/g creatinine). For practical reasons, the assumption has been made that there is no difference between these units, hence the HBGVs expressed as µg/L and µg/g creatinine are the same. However, as reported in section 3, the volume corrected method generally results in higher exposure estimates compared to the creatinine corrected method.

Table 7: Metabolites and HBM-GVs for DEHP and DINCH.

	Metabolites	Metabolites as basis for HBM-GV	HBM I $HBGV_{GenPop}$ adults	HBM I $HBGV_{GenPop}$ children
DEHP	MEHP 5OH-MEHP 5oxo-MEHP	5oxo-MEHP and 5OH-MEHP	500 µg/L	340 µg/L
	5cx-MEPP 2cx-MMHP	5cx-MEPP and 5OH-MEHP	570 µg/L	380 µg/L
DINCH	OH-MINCH cx-MINCH oxo-MINCH	OH-MINCH and cx-MINCH	4500 µg/L	3000 µg/L

6.2.1 DEHP

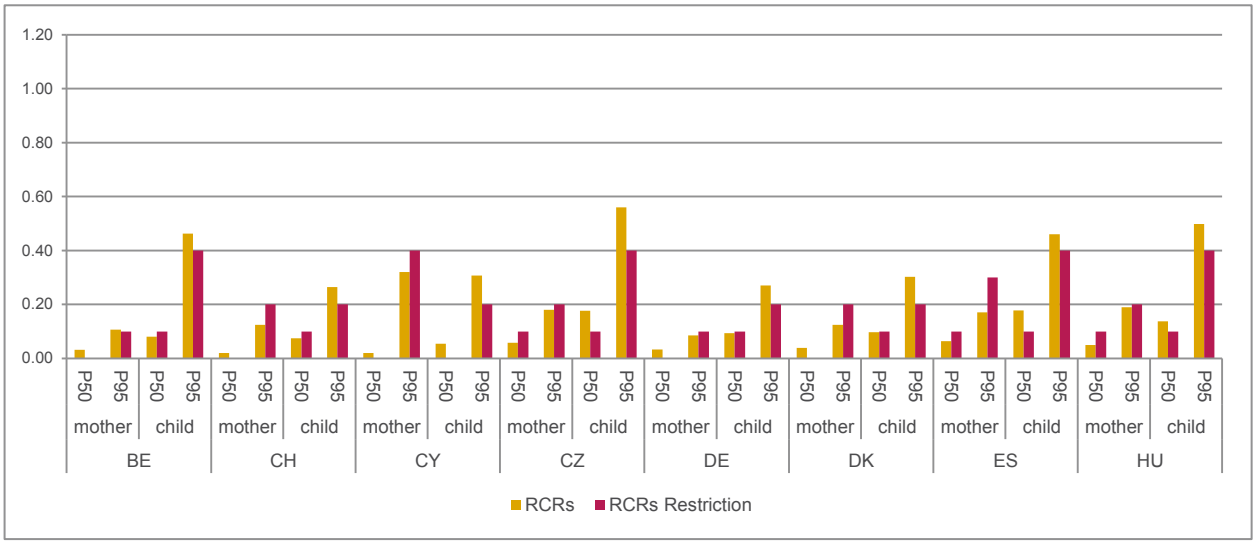
The HBM I $HBGV_{GenPop\ (5\text{-}oxo\text{-}MEHP\ and\ 5OH\text{-}MEHP)}$ for adults was developed within HBM4EU as an alternative to the DNEL derived in the restriction dossier (see section 3). As these guidance values are both based on the NOAEL of 4.8 mg/kg bw/day (Wolfe and Layton, 2003), they would ideally result in the same RCRs, when the same exposure estimates are used. However, this HBM I $HBGV_{GenPop\ (5\text{-}oxo\text{-}MEHP\ and\ 5OH\text{-}MEHP)}$ value cannot be readily compared to the HBM exposure estimates from the restriction, as the data in the restriction dossier are transformed into DEHP intake

estimates. Instead, the underlying data from the DEMOCOPHES project (Den Hond *et al.*, 2014) presented as the metabolite concentrations in urine were used.

Another issue is that the DEHP HBM I HBGV for the general population is presented in µg/L. However, the data available for DEHP metabolites are presented in their creatinine corrected form (i.e. µg/g creatinine). For practical reasons, the assumption has been made that there is no difference between these units, hence the HBGV expressed as µg/L and µg/g creatinine are the same. However, as reported in section 3, the volume corrected method generally results in higher exposure estimates compared to the creatinine corrected method. The outcome of the calculation is presented in **Table 8** and **Figure 2a and b**. From **Table 8** may be concluded that only the RCR of the P50 for mothers changes by using a different calculation method.

Table 8: RCRs resulting from metabolite concentrations in urine from mother-child pairs in the European population (ECHA, 2017a) divided HBM I $HBGV_{GenPop\ (5\text{-}oxo\text{-}MEHP\ and\ 5OH\text{-}MEHP)}$ values, and RCRs as calculated in the Restriction dossier .

Geometric mean EU population	Percentile	MEHP (µg/gC)	5OH-MEHP (µg/gC)	5-oxo-MEHP (µg/gC)	Total 5OH-MEHP and 5-oxo-MEHP (µg/gC)	$HBGV_{GenPop}$ (5-oxo-MEHP and 5OH-MEHP)	RCRs	RCRs restriction
Mother	P50	2.77	12.37	7.73	20.15	500	0.0	0.1
	P95	12.38	51.10	28.94	81.54	500	0.2	0.2
Child	P50	2.66	23.69	15.39	39.16	340	0.1	0.1
	P95	10.52	81.85	49.76	131.96	340	0.4	0.4



From **Figure 3a and b** it may be concluded that there are deviations between the RCRs for the

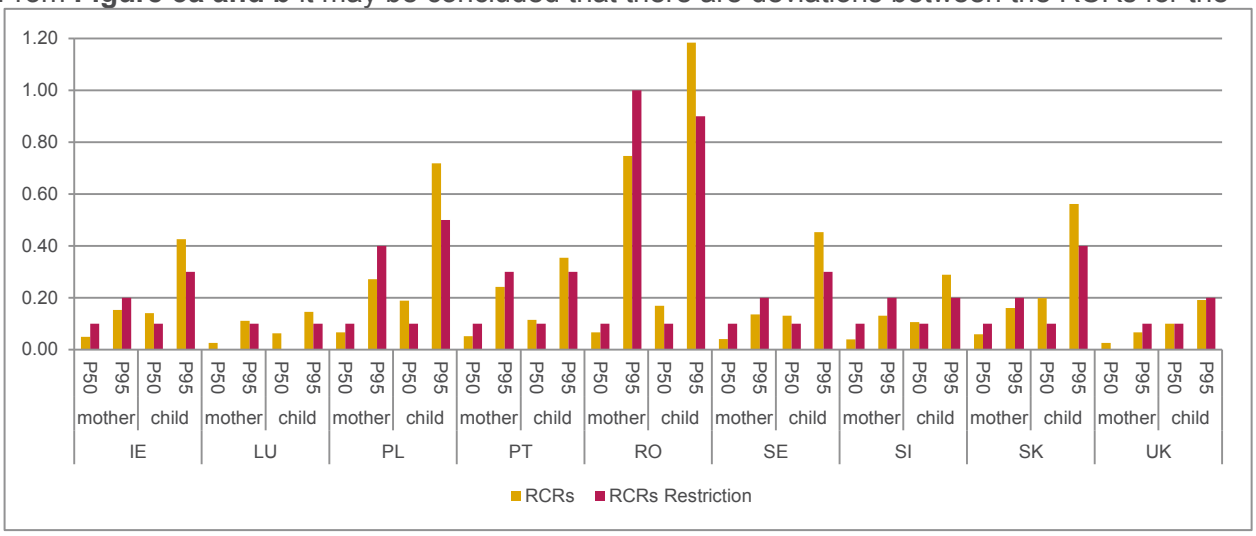


Figure 3a and b: RCRs resulting from creatinine corrected metabolite concentrations in urine from mother-child pairs in the European population (ECHA, 2017a) divided HBM I HBGVGenPop (5-oxo-MEHP and 5OH-MEHP) values, and RCRs as calculated in the Restriction dossier (ECHA, 2017b).

different calculation methods, with overall higher RCRs for children and overall lower RCRs for mothers using the HBM I HBGV compared to the methodology used in the restriction. The only crucial difference is the RCR >1 for the P95 exposure estimates of Romanian children (RCR of 0.9 to 1.2). However, the main conclusion from the restriction dossier was that the risk of exposure to the four phthalates was not adequately controlled, as the RCRs of all four compounds were added up, resulting in substantially higher estimated risks. As there are no HBM I HBGVs derived for DBP, BBP, and DIBP, it is not possible to evaluate whether the HBM based method could have resulted in different overall risk estimates.

6.2.2 DINCH

There are a few publications available that studied DINCH metabolite concentrations in urine (Fromme *et al.*, 2013; Schutze *et al.*, 2014; Giovanoulis *et al.*, 2016). These publications focused on subpopulations (adults, young adults, and children) in Norway and Germany. RCRs resulting from comparison of detected urinary metabolite concentrations to the HBM I $HBGV_{GenPop\ (OH\text{-}MINCH\ and\ cx\text{-}MINCH)}$ for adults of 4.5 mg/L and a HBM I $HBGV_{GenPop\ (OH\text{-}MINCH\ and\ cx\text{-}MINCH)}$ for children of 3 mg/L are presented in **Table 9**. Exposure to DINCH for these subgroups is far below the reference value, and hence no adverse health effects are expected to follow from exposure to DINCH based on the available human biomonitoring data present in the public literature. As the number of studies is limited and represent a rather small number of people, caution should be exercised when interpreting these RCRs.

It should further be noted that the HBGV for DINCH has a low level of confidence and its status is provisional. DINCH has been identified as Substance of Very High Concern (SVHC) under REACH related to endocrine disrupting properties. In vitro and in silico studies showed concerns related to PPAR-alpha antagonism, metabolic disruption, altered expression of a large number of genes involved in major signal transduction pathways and disruption of the androgen-oestrogen homoeostasis. (HBM4EU Deliverable Report 5.2). Another aspect in this regard is thyroid effects, which have been observed and might be interpreted in a different way to date. An EC thyroid workshop compiled the state of the science on thyroid effects (EC, 2017). Therefore, a HBGV level of 3 mg/L and 4.5 mg/L respectively has to be re-assessed in due time especially when exposure levels are increasing.

Table 9: RCRs for exposure to DINCH in several EU subpopulations.

	N	Age (year)	Sampling year	GM OH-MINCH (ug/L)	P95 OH-MINCH (ug/L)	GM cx-MINCH (ug/L)	P95 cx-MINCH (ug/L)	Total (GM)	Total (P95)	HBM-GV	RCR (GM)	RCR (P95)
(Giovanoulis *et al.*, 2016)	61	20-66	2013-2014	0.25	NA	0.23	NA	0.48	NA	4500	0.0	NA
(Schutze *et al.*, 2014)	60	20-30	2012	0.4	2.09	0.18	0.86	0.58	2.95	4500	0.0	0.0
(Fromme *et al.*, 2016)	208	2-7	2011-2012	2.87	9.95	1.89	6.11	4.76	16.06	3000	0.0	0.0

6.3 Time trends

RAC and SEAC acknowledged that human biomonitoring could serve as a public health monitoring tool to study the implementation and effectiveness of the restriction. However, the unknown time lag between withdrawal from the market and the actual exposure reduction, as well as the impossibility to determine the effective reduction of phthalate exposure from a specific set of sources, complicate the monitoring of the impact of the restriction. Despite the difficulty to quantify the effect of the restriction, human biomonitoring could effectively assess the impact of the whole EU legislation regarding phthalates (ECHA, 2017b).

Despite the latter mentioned limitations, a first attempt was made to estimate the effectiveness of the restriction in the restriction proposal (D-EPA, 2016). Danish and German data from 2002-2011 were compared to the calculated intake estimates from the DEMOCOPHES project. From this was concluded that over course of the years 2001-2011, exposure to DEHP, DBP and BBP was

reduced between 30% and 80%. It was assumed that the declining trend would continue after 2011, as illustrated in (Koch *et al.*, 2017). Therefore, P95 internal exposure estimates determined in the DEMOCOPHES project were extrapolated to the years 2014, 2020 and 2039, both in absence and in presence of a restriction, to project the risks for the future. However, as these time trends were observed in homogeneous subpopulations in Germany and Denmark, it was stressed in (ECHA, 2017b) that the extent of decline was not representative for the non-homogeneous European population, nor for all European countries.

6.3.1 Time trends of restricted phthalates (DEHP, BBP, DBP & DiBP)

The sum of the five metabolites MEHP, 5OH-MEHP, 5oxo-MEHP, 5cx-MEHP and 2cxMEHP together reflect the exposure to DEHP. Several studies have reported a decreasing temporal trend for DEHP metabolites (Gyllenhammar *et al.*, 2017; Koch *et al.*, 2017; Schoeters *et al.*, 2017) between 2009-2014, 2007-2014 and 1990-2015 respectively. Also concentrations of the metabolites of other phthalates MnBP, MiBP, and MBzP (representing DnBP, DiBP, and BBzP) significantly decreased over time (Gyllenhammar *et al.*, 2017; Koch *et al.*, 2017; Schoeters *et al.*, 2017). Koch *et al.* (2017) report a tenfold decrease of DnBP between 1988-2015 (from 100 µg/L in 1988 to 10 µg/L in 2015) and Schoeters *et al.* (2017) observed 40% decrease of DnBP between 2007-2015. MBzP concentrations also significantly declined between 1990-2015, in parallel to DnBP (Koch *et al.*, 2017). In contrast, Koch *et al.* (2017) observed no temporal trend for MiBP between 1988 and 2011. Only during the last years, a decline set in for this substance as well.

Several regulatory measurements have been taken to restrict the production and use of phthalates, such as restricted use in toys and childcare under REACH Regulation (EC) No. 1907/2006, classification of phthalates as Reproductive Toxicity 1B under CLP Regulation (EC) No. 1272/2008[22], a ban in the use of phthalates under Cosmetics Regulation (EC) No. 1223/2009, restricted use in food contact materials in 2011 (Regulation (EC) No. 10/2011), and amendment to the restricted use under REACH Regulation (EC) No. 1907/2006 in 2015. The decreasing trends illustrated above show that efforts of the EU to phase out the production and use of DEHP, BBzP, DnBP, and DiBP have resulted in decreased human exposure to these phthalates.

6.3.2 Time trends of alternative phthalates (DEP, DMP, DiNP, DiDP/DPHP)

As with the restricted phthalates, also for the alternative phthalate DEP a decreasing time trend was observed (Gyllenhammar *et al.*, 2017; Koch *et al.*, 2017). Koch *et al.* (2017) reported levels of the metabolite MEP declining from 54 µg/L in 2007 to 14 µg/L in 2015. Also levels of DMP have decreased, as metabolite levels of MMP were reduced from around 8 µg/L in 2007 to 3 µg/L in 2015 (Koch *et al.*, 2017). In contrast, concentrations of the three metabolites of DiNP (OH-MiNP, oxo-MiNP and cx-MiNP) showed no overall temporal change (Gyllenhammar *et al.*, 2017; Koch *et al.*, 2017). Koch *et al.* (2017) observed a small increase in OH-MiNP from 1.5 µg/L in 1988 to 3 µg/L between 2002 and 2008, but a lower value was detected in 2015 again (2.4 µg/L). This should be considered a worrisome finding, as phthalates act via the same MoA and therefore DiNP contributes to phthalate mixture toxicity. Koch *et al.* (2017) report increased values of DHPH between 1999 and 2012, however these values do not exceed 1 µg/L. Since the analytical method cannot differentiate between metabolites of DPHP and OH-MiDP, this value both represents exposure to DiDP and DPHP. The overall decrease in phthalate exposure in 2015 may be due to

[22] It should be noted that some phthalates might not be classified for reproductive toxicity, but still they would add to mixture toxicity as they act via the same MoA. However, their potency was less and the effects were not sufficiently adverse to support a classification, e.g. DiNP.

producers already anticipating on the phthalates restriction, for which already in 2012 a first restriction proposal was written. This may have encouraged them to replace all phthalates, not only those included in the restriction.

6.3.3 Time trend for the alternative plasticizer DINCH

DINCH was introduced in 2002 as replacement for high molecular weight phthalates in PVC, such as DEHP. For this alternative, a considerable increase in exposure has been observed over time (Schutze *et al.*, 2014; Gyllenhammar *et al.*, 2017). Between 2009 and 2014 concentrations of a metabolite of DINCH, oxo-MINCH, were significantly increased, with an adjusted mean urine concentration from 0.2 to 0.7 µg/L (Gyllenhammar *et al.*, 2017). Also Schutze *et al.* (2014) reported an increase in concentration between 1999 and 2006. In the years 1999 and 2003, no OH-MINCH, cx-MINCH, and oxo-MINCH (three metabolites of DINCH) could be detected. Between 2006 and 2012, the concentration of OH-MINCH, cx-MINCH, and oxo-MINCH increased significantly from <LOQ (<LOQ; 0.09 µg/L) to 0.4 µg/L (0.39 µg/L; 2.09 µg/L), <LOQ (<LOQ, <LOQ) to 0.18 µg/L (0.17 µg/L; 0.86 µg/L), and <LOQ (<LOQ; 0.05 µg/L) to 0.25 µg/L (0.25 µg/L; 1.81 µg/L) respectively. For the monoester of DINCH (MINCH), the detection rates were too low to observe any trends in time.

Although the number of studies that include DINCH is still limited, the available HBM data suggest an increase in the exposure to DINCH in last years. However, the exposure levels in the most recent measurements were still far below the HBM-GV. However, as noted above, the HBGV for DINCH has a low level of confidence and its status is provisional. Therefore, in due time the HBM-GVs needs to be re-assessed, especially when exposure levels are increasing.

7 Conclusion

Human biomonitoring data was successfully used in the restriction dossier as a reference to determine whether modelled exposure estimates provide a realistic representation of the actual internal exposure. The human biomonitoring data provided an overall estimation of exposure, but cannot be used to determine the contribution of different sources to the overall exposure. There are various factors that induce uncertainty when using RCRs based on human biomonitoring data, such as the variation in the methodology to determine the phthalate metabolite concentration in urine, limited correction for the age of the children sampled, the exclusion of specific groups in the DEMOCOPHES study (i.e. haemodialysis patients), and the relatively small sample size for specific EU countries. It is additionally illustrated in this document that the RCR values strongly depend on the methodology used to express hazard and exposure (i.e. internal concentrations or urinary metabolite concentrations).

Furthermore, there is potential for human biomonitoring to assess the impact of the whole EU legislation regarding phthalates retrospectively (ECHA, 2017b). The available data (including monitoring data up until 2015) show an overall declining trend in the use of phthalates with the exemption of DiNP, while the alternative phthalates DiDP and DPHP, and the alternative plasticiser DINCH show an increasing trend. To observe if the restriction has a positive impact in the effective reduction of the exposure to phthalates, data from 2017 and onwards must be available (preferably on an EU wide scale). HBM4EU therefore may play a valuable role in monitoring the effectiveness of chemicals legislation, including the phthalates restriction. We however stress that the current calculations were an exercise to include HBM data and that the output should not yet be used by policymakers. Despite important, these results should be cautiously interpreted due to the assumptions and uncertainties considered.

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Annex G: phthalates risk assessment (part B: occupational population)

Part B: D5.5 Substance-group specific risk assessment for phthalates concerning occupational exposure

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1 Introduction

Risk assessment for three phthalates: diisononyl phthalate (DiNP), diisodecyl phthalate (DiDP) and di(2-propylheptyl) phthalate (DPHP), was carried out regarding occupational exposure. These three phthalates were included since their use has not been restricted, and they are widely used in plastic product manufacturing.

2 Methodology

To our knowledge, occupational risk assessments for DiNP, DiDP or DPHP have not been carried out yet. Therefore, a risk assessment using human biomonitoring (HBM) data for the exposure assessment was performed.

Occupational exposure limit (OEL) values have not been derived for DiNP, DiDP or DPHP, neither for internal nor external exposure. However, for DPHP, there is a provisional EU HBM guidance value (HBM-GV) available for the occupational population, under revision in the task 5.2 of HBM4EU at the time of writing this deliverable.

For DiNP and DiDP, a simple one-compartment model biomonitoring equivalent (BE) methodology was applied to derive HBM assessment values (Angerer *et al.*, 2011), as other means of relating the external and internal exposure levels (such as PBPK modelling or conversion equations) were unavailable. The BE approach was based on previously derived external tolerable intake values, in this case DNELs for the general population derived by ECHA (ECHA, 2013d), which were used to calculate occupational DNELs for exposure via inhalation.

There are earlier BEs for the metabolites of DiNP for the general population (Hays *et al.*, 2011). They are based on health-based reference values by Health Canada, the US Consumer Product Safety Commission and the European Food Safety Authority, which are higher than the ECHA DNELs used here. Also the resulting earlier BEs by Hays et al. are higher (~2–6x) than those calculated for the general population here. For the current derivation of BEs, the latest, European health-based reference values, i.e. the DNELs by ECHA, were regarded as the most relevant.

For the exposure assessment, published HBM data from the literature was used.

3 Summary of hazard characterisation

The most prominent health concerns related to phthalates are their endocrine disrupting properties and adverse effects on reproduction, which are mediated through the anti-androgenic mode of action (Mariana *et al.*, 2016). The use of several phthalates has been restricted under the REACH regulation, based on reproductive toxicity in laboratory animal species (see the general population section, Chapter 3, presented above). The observed

adverse effects include abnormalities in the rat male offspring exposed during the critical time window of gestation, for example malformations of the testes and epididymides, cryptorchidism, hypospadias and reduced semen count (Bundesgesundheitsbl, 2011). The modes of action include disruption of the development of Leydig cells, reduced or inhibited testicular testosterone production and reduced production of the insulin-like 3 peptide hormone. Similar adverse effects are also assumed in humans, and an association between them and phthalate exposure has been shown in several studies (Jurewicz and Hanke, 2011), although a causal link is yet to be established in epidemiological studies.

However, not all phthalates induce reprotoxic effects, and their endocrine disruption potencies vary due to differences in their molecular structures. Compared with the phthalates considered to be the most potent, DiNP appears to have a somewhat lower endocrine disrupting potency (ECHA, 2013d; Gennings *et al.*, 2014), while DiDP is considered to not have substantial anti-androgenic effects (ECHA, 2013d). Based on its molecular structure, the same is thought for DPHP, but experimental data is still mostly lacking. However, DiDP has been shown to interfere with development during gestation, inducing retardation of growth, developmental disorders and lethality at high doses in rats, although these effects are suggested not to be mediated by the antiandrogenic mode of action (ECHA, 2013d). DPHP appears to not induce direct reproductive toxicity in rats, but has adverse effects on several organs, including the thyroid, via a mechanism that is at present unclear (Bhat *et al.*, 2014). Both DiNP and DiDP have hepatotoxic effects in rats following repeated exposure.

4 Summary of available risk assessments

To our knowledge, occupational risk assessments have not been performed for DiNP, DiDP or DPHP.

5 Risk assessment based on human biomonitoring data

5.1 Hazard assessment and derivation of BEs

DiNP and DiDP:

As OELs have not been set for DiNP or DiDP, we used as a starting point DNELs derived for general population by the European Chemicals Agency (ECHA) in 2013 [Table 1. Repeated dose DNELs for adult populations for (ECHA, 2013d)]. These were converted to worker DNELs following the standard approaches described in the REACH guidance R.8 (ECHA, 2012b), taking into account differing exposure times (8 h/day and 5 days per week for workers), and smaller intraspecies variation compared to the general population.

ECHA derived two DNELs for both DiNP and DiDP, based on reprotoxic and hepatotoxic effects. Of these, hepatotoxicity was considered the critical effect, leading to slightly lower DNELs than reprotoxicity for both phthalates. For the derivation of the general population DNELs for hepatotoxicity, ECHA used repeated dose studies. For DiNP, this was a 2-year oral study in rats with a NOAEL of 15 mg/kg bw/day, leading to an oral DNEL of 0.075 mg/kg/day, and an inhalation DNEL of 0.35 mg/m^3/day (ECHA, 2013d)(Table 1). For DiDP, a weight of evidence approach was selected by ECHA, and three repeated-dose studies were considered together: a 2-year study in rats (LOAEL 22 mg/kg bw/day), a 90-day study in dogs (NOAEL 15 mg/kg bw/day) and a 90-day study in rats (NOAEL 60 mg/kg bw/day) (ECHA, 2013d). Considering the three DNELs derived from these studies, the average values lead to the same oral and inhalation DNELs for repeated dose toxicity as for DiNP (Table 1).

Occupational DNELs were not derived by ECHA, so they were derived here based on the same critical effects as presented above and standard assumptions and assessment factors as used by ECHA previously for the derivation of occupational DNELs for DBP, BBP and DEHP (ECHA, 2013a; ECHA, 2013b; ECHA, 2013c). The first calculation, for deriving a human equivalent point of departure (POD), was based on the same NOAEL of 15 mg/kg bw/day as used by ECHA in the derivation of the general population DNELs. It was multiplied with the following factors to correct for several differences:

- 1/0.38 for the standard respiratory volume in m^3/kg bw per day
- 50/75 for the 50% oral absorption in rats and assumed 75% inhalation absorption in adult humans
- 6.7/10 to account for increased breathing volume (m^3) from rest to light work
- 7/5 for a shorter exposure duration in the occupational vs. general population (7 vs. 5 days a week),

The resulting human equivalent POD (25 mg/m^3), was further corrected with the assessment factors of 2.5 (for interspecies differences in toxicodynamics) and 5 (for intraspecies variation, which is assumed smaller in the occupational vs. the general population)(ECHA, 2012b), resulting in an occupational inhalation DNEL 2.0 mg/m^3 (Table 1). The corresponding inhaled dose as mg/kg was calculated by multiplying the inhalation DNEL with a factor of 10 (to account for the average breathing volume of 10 m^3 during a working day), and dividing it by 70 (assumed average body weight of workers), resulting in 0.28 mg/kg (8 h TWA).

Table 1: Repeated dose DNELs for adult populations for DiNP and DiDP.

CAS number(s)	Phthalate	General population		Occupational population	
		DNEL, oral (mg/kg/day)	DNEL, inhalation (mg/m^3/day)	DNEL, inhalation (mg/m^3, 8 h TWA)	Inhaled dose as mg/kg (8 h TWA)
28553-12-0 / 68515-48-0	DiNP	0.075 [a]	0.35 [a]	2.0 [b]	0.28 [b]
68515-49-1 / 26761-40-0 [c]	DiDP	0.075 [a]	0.35 [a]	2.0 [b]	0.28 [b]

[a] Reference DNELs by ECHA (2013d) (critical effect on the liver)

[b] Derived by the authors using the same assumptions as ECHA previously

[c] Not registered under REACH

Finally, the one-compartment BE methodology (Angerer *et al.*, 2011) was applied to roughly estimate the urinary levels of the three main DiNP metabolites at steady state (C_{ss}), corresponding to the external DiNP intake levels. For the calculation, the following formula was used:

$$Css = \frac{\mathrm{D} \times \mathrm{BW} \times \mathrm{Fue}}{\mathrm{V24}}$$

where D is the inhaled (external) dose as mg/kg bw, F_{UE} is the mass-based factor of metabolite excreted to the urine during 24 hours (per mass of parent compound ingested), and V_{24} is the estimated average 24-hour urinary volume (assumed as 1.5 L) (Angerer *et al.*,

2011). Mean bodyweight (BW) was assumed as 70 kg. The 24 h F_{UE} values (calculated as mass-fraction) used for the DiNP metabolites OH-MiNP, oxo-MONP and cx-MiNP were 0.084, 0.046 and 0.076, respectively (Table 2).

Based on these calculations, the BEs for the occupational population (for the three DiNP metabolites) are presented in Table 2. Also the BEs for the general population are included. The external doses used in their calculation were the oral DNELs of 0.075 mg/kg/day derived by ECHA (ECHA, 2013d).

For DiDP, the resulting BEs would be identical due to the same DNEL, as the F_{UE} values would have to be assumptions based on DiNP, as DiDP-specific data is not available. This approach is considered the best available and reasonably justified, as DiNP and DiDP structures are very similar to each other (DiNP includes once carbon atom more). Also ECHA and an earlier EU risk assessment (reviewed in the ECHA assessment) considered DiNP and DiDP analogous regarding toxicokinetics (ECHA, 2013d).

Table 2: The calculated BEs for the three DiNP metabolites, and their fractional 24-h excretion values (mole basis and calculated as mass fraction).

Metabolite	BE (mg/L) Occupational population	BE (mg/L) General population	Molar excretion (%) of 24-h DiNP dose, mean ± SD (Anderson *et al.*, 2011)	F_{UE} (%), mass excretion of 24-h DiNP dose[a], mean ± SD
OH-MiNP	1.10	0.29	11.4 ± 3.26	8.4 ± 2.4
oxo-MiNP	0.60	0.16	6.3 ± 1.81	4.6 ± 1.3
cx-MiNP	1.00	0.27	9.9 ± 3.00	7.6 ± 2.3

[a] Calculated as ratio of molecular weight of metabolite over DiNP times the molar excretion percent.

DPHP:

For DPHP, a new provisional EU HBM-GV has been derived in task 5.2 for workers: 0.9 mg/L for the urinary metabolite OH-MPHP at the end of the work shift. This value is still under review and was used here only as indicative.

5.2 Evaluation of the available biomonitoring data

Table 3 presents the biomonitoring data of occupational exposure to DiNP, DiDP and DPHP. Data on occupational DiNP exposure consist of six studies. In three of these studies, the primary metabolite (hydrolytic monoester) mono-isononylphthalate (MiNP) has been analysed. However, MiNP is not a reliable metabolite for DiNP exposure because it is prone to external DiNP contamination and because it is extensively further metabolised to secondary metabolites (oxidised monoesters) of DiNP (Koch *et al.*, 2007). Indeed, the percentage of MiNP of the total amount of urinary excretion is low (~2%) (Koch and Angerer, 2007). Thus, the secondary metabolites of DiNP, which are not influenced by contamination, are more reliable markers for the DiNP exposure. Urinary biomonitoring data on secondary DiNP metabolites, namely OH-MiNP, oxo-MiNP and cx-MiNP, were selected for further discussion.

Three occupational studies exist where the secondary metabolite cx-MiNP has been analysed in post-shift samples (Table 3). Geometric mean (GM) urinary cx-MiNP concentration of seven workers who used DiNP on PVC film manufacturing in USA was 50.5 µg/L (data range 15-164 µg/L) (Hines *et al.*, 2012). The respective GM of those 11 workers

who worked in the same shift but were not in direct contact with DiNP was 34,1 µg/L (data range 12.2-164 µg/L). With both group of workers there was a high significant difference in urinary cx-MiNP results when compared to control group (in both cases p<0.0001). Hines et al. also studied exposure of 12 workers in PVC compounding. Their post-shift urinary results (GM 9.05 µg/L and data range 1.86-19.8 µg/L) were statistically different compared to mid-shift results of the same workers (p=0.03). These results prove occupational DiNP exposure of the PVC workers.

Koch et al. have studied five PVC plastisol workers in Germany (Koch *et al.*, 2012). Urinary median cx-MiNP concentration was 57.8 µg/L and the data range 24.7-286 µg/L. Statistical comparison was not given but concentrations in post-shift samples where approximately 20-times higher than the results of the general population indicating occupational exposure.

In a third occupational DiNP study Porras et al. studied workers in PVC plastic manufactory and PVC coated textile manufactory in Finland (Porras *et al.*, 2016). No statistical comparison was made because of small number of workers in both cases (Table 3). After visual inspection of the urinary cx-MiNP concentrations of PVC plastic workers (GM 7.6 µg/L, median 6.4 µg/L, range 3.7-16.8 µg/L), the results are roughly in the same range than those of the non-occupationally exposed control group (n=60). The respective results of PVC coated textile workers (GM 5.5 µg/L, median 5.9 µg/L, range <LOQ-49.3 µg/L) are in also the same range than those of the control group. But in the latter group, the maximum concentration of the workers is slightly higher than the 95th percentile of the control group (32.3 µg/L). This indicates a low level of occupational exposure.

Koch et al. have also analysed other secondary metabolites of DiNP (OH-MiNP and oxo-MiNP, Table 3) (Koch *et al.*, 2012). The conclusions are the same as with cx-MiNP (see above): workers were occupationally exposed to DiNP.

The data on occupational DiDP and DPHP exposure are scarce. Koch et al. have studied DiDP metabolites of PVC plastisol workers exposed to DiNP (Koch *et al.*, 2012). They analysed also other phthalates, like DiDP, because isomeric DiNP used in the factory can contain to some extent other high molecular weight phthalates. Urinary concentrations of OH-MiDP (median 16.8 µg/L, range 5.6-57.6 µg/L), oxo-MiDP (median 4.6 µg/L, range 1-12.1 µg/L) and cx-MiDP (median 4.7 µg/L, range 2.1-20.3 µg/L) were higher than the respective data of the general population in Germany (median 0.7 µg/L, range <LOQ-3.4 µg/L; median 0.3 µg/L, range <LOQ-1.9 µg/L; median 1.0 µg/L, range <LOQ-5.7 µg/L, respectively). No statistical comparison was made. Note. The analytical separation method used in the work could not differentiate the secondary metabolites of DiDP and DPHP (or other C10-phthalates), which means that the source of exposure might have been either DiDP or DPHP.

Gries et al. (2012) have worked out on this matter by re-analyzing 12 individual urine samples of plastisol workers taken from the study of (Koch et al. (2012). They used GC–HRMS technique which allows to distinguish between DiDP and DPHP metabolites due to higher separation power compared to LC-MS/MS technique. The results show that the amount of DPHP metabolites was low – only oxo-MPHP could be detected (range <LOQ-0.72 µg/L).

Porras and co-workers have studied the DPHP exposure of factory workers producing PVC cables where DPHP was used as a plasticiser. The urinary OH-MPHP results of post-shift samples were: GM 3.5 µg/L, median 3.9 µg/L and the range 1.8-7.8 µg/L. The results of the control group (n=60) were all <LOQ. Accordingly the results indicate a low level of

occupational exposure to DPHP. Also in this case, the analytical separation method used was not capable of differentiating the exposure to DiDP and DPHP. So the measured low urinary cx-MiDP concentrations of the same PVC cable workers (Table 3) could also be due to DPHP exposure.

In the above-mentioned study of DiNP exposure in PVC plastics factory, also DPHP was used in the production (Porras et al., 2016). Workers' post-shift urinary OH-MPHP concentrations were: GM 4.7 µg/L, median 7.1 µg/L and range <LQQ-21.0 µg/L (Table 3). Also in this case, low OH-MPHP concentrations indicate a small scale occupational exposure because the respective concentrations of the control group (n=60) were all below LOQ.

As a conclusion, occupational biomonitoring studies on DiNP, DiDP and DPHP exposure are scarce (Table 3). There are a few studies that indicate that PCV plastics workers are more exposed to DiNP than the reference populations. The reported post-shift urinary concentrations of the DiNP metabolite OH-MiNP, are in the range of 59.3-443 µg/L. The respective oxo-MiNP concentrations are in the range of 10.7-175 µg/L, and cx-MiNP concentrations are in the range of <1.0 µg/L-286 µg/L. Some data exists on PVC workers exposure to DiDP/DPHP. The reported post-shift urinary concentrations of the DiDP metabolites are 5.6-57.6 µg/L (OH-MiDP). 1.0-12.1 µg/L (oxo-MiDP), and <1.0-20.3 µg/L (cx-MiDP). The post-shift concentrations of OH-MPHP (metabolite of DPHP) are <1.0-21.0 µg/L. However, the data on exposure to DiDP/DPHP (or other C10-phthalates) should be interpreted with caution especially when the analytical separation method used is not capable of separating the metabolites of these phthalates.

Table 3: Biomonitoring results on occupational exposure to DiNP, DiDP and DPHP. Units µg/L.

Study	Country	Parent compound	Metabolite	LOD / LOQ	Job / task	n	GM	Median	P95	Range
(Kolena *et al.*, 2014)	Slovakia	DiNP	MiNP	8.12 / -	Waste management workers	30	-	<LOD	14.89	-
(Petrovičová *et al.*, 2014)	Slovakia	DiNP	MiNP	8.12 / -	Plastic manufactory workers	37	-	13.65	21.9	4.6-23.37
(Pilka *et al.*, 2015)	Slovakia	DiNP	MiNP	8.12 / -	Community service workers	48	-	4.6	14.89	-
(Pilka *et al.*, 2015)	Slovakia	DiNP	MiNP	8.12 / -	Plastic manufactory workers	37	-	13.65	21.74	-
(Koch *et al.*, 2012)	Germany	DiNP	cx-MiNP	- / 0.25	PVC plastisol workers	5	-	57.8	-	24.7-286
(Hines *et al.*, 2012)	USA	DiNP	cx-MiNP	0.7 / -	PVC film manufacturing, DiNP used on worker's task	7	50.5	-	-	15-164
(Hines *et al.*, 2012)	USA	DiNP	cx-MiNP	0.7 / -	PVC film manufacturing, DiNP used on worker's shift	11	34.1	-	-	12.2-164
(Hines *et al.*, 2012)	USA	DiNP	cx-MiNP	0.7 / -	PVC compounding	12	9.05	-	-	1.86-19.8
(Porras *et al.*, 2016)	Finland	DiNP	cx-MiNP	- / 1.0	PVC plastic manufactory workers	5	7.6	6.4	16.3	3.7-16.8
(Porras *et al.*, 2016)	Finland	DiNP	cx-MiNP	- / 1.0	PVC coated textile workers	5	5.5	5.9	40.3	<LOQ-49.3
(Koch *et al.*, 2012)	Germany	DiNP	OH-MiNP	- / 0.25	PVC plastisol workers	5	-	117	-	59.3-443
(Koch *et al.*, 2012)	Germany	DiNP	oxo-MiNP	- / 0.25	PVC plastisol workers	5	-	44.3	-	10.7-175
(Koch *et al.*, 2012)	Germany	DiDP/DPHP [a]	cx-MiDP	- / 0.25	PVC plastisol workers	5	-	4.7	-	2.1-20.3
(Porras *et al.*, 2016)	Finland	DiDP/DPHP [b]	cx-MiDP	- / 1.0	PVC cable factory workers	5	1.9	2.5	6.7	<LOQ-7.5
(Koch *et al.*, 2012)	Germany	DiDP/DPHP [a]	OH-MiDP	- / 0.25	PVC plastisol workers	5		16.8	-	5.6-57.6
(Porras *et al.*, 2016)	Finland	DiDP/DPHP [b]	OH-MPHP	- / 1.0	PVC cable factory workers	5	3.5	3.9	7.2	1.8-7.8
(Porras *et al.*, 2016)	Finland	DiDP/DPHP [b]	OH-MPHP	- / 1.0	PVC plastic manufactory workers	5	4.7	7.1	19.3	<LOQ-21.0
(Koch *et al.*, 2012)	Germany	DiDP/DPHP [a]	oxo-MiDP	- / 0.25	PVC plastisol workers	5	-	4.6	-	1.0-12.1

[a] The analytical separation method used could not differentiate the secondary metabolites of DiDP and DPHP (or other C10-phthalates), which means that the source of exposure might have been either DiDP or DPHP. During the measurement day, DiNP was used, but it may have contained also DiDP and DPHP.
[b] The analytical separation method used could not differentiate the secondary metabolites of DiDP and DPHP (or other C10-phthalates), which means that the source of exposure might have been either DiDP or DPHP. However, during the measurement day, only DPHP was used.
LOD = Limit of detection, LOQ = Limit of quantitation

5.3 Risk assessment for the sample populations

DiNP and DiDP:

The DiNP metabolite cx-MiNP was considered here for risk assessment, as HBM measurement data is available for it from three separate studies (n=45), while for the two other metabolites, data is only available for one study for each metabolite (n=5 in both). Thus, the data for cx-MiNP was considered the most reliable. In addition, it is one of the two main secondary metabolites of DiNP, with a mass-corrected elimination fraction of 7.6% of the DiNP dose (within 24 h). Also for the two other metabolites (oxo- and OH-MiNP), the resulting RCRs would be similar (calculations not shown).

Exposure assessment: Based on the available HBM data, the reported post-shift urinary concentrations for the respective DiNP and DiDP metabolites are <1.0 to 286 µg/L (cx-MiNP) and <1.0 to 20.3 µg/L (cx-MiDP). Maximum values were used for the RCR calculations, representing worst case scenarios concerning the available data.

Hazard assessment: Using the BE approach, based on the DNEL values, the calculated BE for DiNP (cx-MiNP) is 1.0 mg/L for the occupational population (Table 2). The same BE is assumed for DiDP (cx-MiDP). For comparison, for the general population, the respective BEs are approximately four-fold lower (Table 2).

Risk characterisation: Using the maximum measured urinary concentrations, the occupational RCRs for DiNP and DiDP are well below 1, indicating a low risk of adverse health effects based on conservative assumptions:

RCR_{DINP}: 286 µg/L / 1000 µg/L = 0.29

RCR_{DIDP}: 20.3 µg/L / 1000 µg/L = 0.02

DPHP

DPHP is presented here only as indicative, as the new provisional EU HBM-GV is still under review, and also the HBM data for DPHP is very scarce.

Exposure assessment: Based on the available HBM data, the reported posts-shift urinary concentrations for the DPHP metabolite OH-MPHP are <1.0–21.0 µg/L. For the other DPHP metabolites, such as oxo-MPHP, HBM data are not available.

Hazard assessment: The provisional EU HBM-GV for DPHP (OH-MPHP) is 0.9 mg/L.

Risk characterisation: The urinary concentration of the DPHP metabolite OH-MPHP is roughly 40x lower than the provisional EU HBM-GV of 0.9 mg/L, indicating a low occupational risk for this individual phthalate, based on conservative assumptions. However, this approach is presented merely as indicative, and includes several uncertainties stemming particularly from lack of data. In addition, the provisional EU HBM-GV is still under revision.

6 Discussion and conclusions

There are some uncertainties related to the risk assessment of DiNP and DiDP performed here. Several assumptions have been made based on lack of available data, particularly for DiDP. Also, extrapolating the human POD from animal studies includes several approximations. Moreover, for the exposure assessment, maximum measured urinary values were used instead of for example P95 values. This was due to a) the P95 not being available for all of the studies, and b) the small n in each study. All of these aspects are likely to bring considerable conservativeness to the risk assessment.

It should be also noted that the C_{ss} value in the one compartment model based BE equation corresponds to the urinary level of a substance at steady state. As both DiNP and DiDP are excreted rather rapidly (Anderson *et al.*, 2011), and the urinary measurements were taken at the end of the work shift, they probably represent peak values rather than steady state. This is likely to bring additional conservativeness to the assessment. 24-h urine samples would give more reliable results, but in practice, these kinds of samples are not usually available due to practical reasons regarding the study design. Repeated spot samples could help to define the degree of the uncertainty related to the use of single (post-shift) spot samples.

However, even based on the relatively conservative risk assessment performed here, the occupational risk for DiNP- and DiDP-related adverse health effects appears low. Although the simple one-compartment model BE approach is quite rough, giving only an estimate of a health-based reference value, it may in many cases be considered sufficient for an approximate risk assessment. For example, in this case, the differences between the occupational BEs and measured phthalate levels are so large, that this kind of rough estimation can demonstrate low risk for these individual phthalates. On the other hand, it is known that mixtures of phthalates have direct additive effects (Howdeshell *et al.*, 2017) and it is not uncommon for workers to be exposed to phthalate mixtures. This should be considered in further risk assessments.

Additionally and very importantly, the HBM data on occupational exposure to DiNP, DiDP and DPHP is very limited, covering only a limited range of tasks and geographical area. Therefore, the representativeness of the data is uncertain. Furthermore, the exposure data for DiDP and DPHP should be interpreted with caution as the analytical separation method used in the available studies was not capable of separating the metabolites of these phthalates.

On a general level, HBM can be considered a particularly important tool for the exposure assessment of phthalates, as they are not particularly volatile. Thus, their air concentration measurements can be misleading, and exposure via inhalation may not be the only significant route, making consideration of exposure via also the other exposure routes important.

6.1 Future prospects

There is relatively much occupational exposure data available for the already restricted phthalates. For the newer phthalates that are being used to replace them, more occupational exposure data is needed, since currently the database is very limited. Moreover, as the use of these newer phthalates will likely increase and the exposure levels will subsequently rise in the general population, distinguishing low occupational exposures will become more difficult.

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