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How to use human biomonitoring in chemical risk assessment: Methodological aspects, recommendations, and lessons learned from HBM4EU

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ABSTRACT

One of the aims of the European Human Biomonitoring Initiative, HBM4EU, was to provide examples of and good practices for the effective use of human biomonitoring (HBM) data in human health risk assessment (RA).

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The need for such information is pressing, as previous research has indicated that regulatory risk assessors generally lack knowledge and experience of the use of HBM data in RA. By recognising this gap in expertise, as well as the added value of incorporating HBM data into RA, this paper aims to support the integration of HBM into regulatory RA. Based on the work of the HBM4EU, we provide examples of different approaches to including HBM in RA and in estimations of the environmental burden of disease (EBoD), the benefits and pitfalls involved, information on the important methodological aspects to consider, and recommendations on how to overcome obstacles. The examples are derived from RAs or EBoD estimations made under the HBM4EU for the following HBM4EU priority substances: acrylamide, o-toluidine of the aniline family, aprotic solvents, arsenic, bisphenols, cadmium, diisocyanates, flame retardants, hexavalent chromium [Cr(VI)], lead, mercury, mixture of per-/poly-fluorinated compounds, mixture of pesticides, mixture of phthalates, mycotoxins, polycyclic aromatic hydrocarbons (PAHs), and the UV-filter benzophenone-3. Although the RA and EBoD work presented here is not intended to have direct regulatory implications, the results can be useful for raising awareness of possibly needed policy actions, as newly generated HBM data from HBM4EU on the current exposure of the EU population has been used in many RAs and EBoD estimations.

1. Introduction

The Horizon 2020 co-financed European Human Biomonitoring Initiative HBM4EU (2017–2022; www.hbm4eu.eu) was set up to coordinate and advance human biomonitoring (HBM) in Europe. Its main aim was to support policy-making by providing better evidence of the actual internal exposure of populations to chemical substances and mixtures, and to link this exposure to possible adverse health effects (Ganzleben et al., 2017). This included also providing examples of and good practices for effectively using HBM data in regulatory human health risk assessment (RA) and decision-making.

HBM is an important tool for assessing exposure to chemicals and their health risks. It provides information on aggregated and actual internal exposure, accounting for combined different exposure sources, and all exposure routes and interindividual differences in, for example, metabolism, diet, and lifestyle (Angerer et al., 2007). As such, it supports the new EU chemicals strategy for sustainability, promoting the 'one substance, one assessment' principle (EC, 2020). As HBM measures the actual concentrations of chemicals or their metabolites in the human body (internal exposure), it complements external exposure assessments and provides useful information for assessing health risks. The drawback of applying HBM data in RA is the limited guidance available for interpreting the biomarker levels concerning adverse health effects and for linking internal exposure levels to external intake/exposure.

In addition to being valuable for RA, HBM also offers insights into the time trends of the total internal exposure of a substance or its metabolite, when it has been/is performed on a regular basis (Kolossa-Gehring et al., 2012). In this way, it provides information on the effectiveness of existing risk management measures and helps identify further regulatory needs. For example, the current urinary levels of restricted phthalates in the European general population are clearly decreasing, but novel substitutes are increasing (Lemke et al., 2021). Furthermore, HBM data can provide information on the exposure of subpopulations, distinguished by, for example, country, age, sex, diet, behaviour, or socioeconomic status (SES). This knowledge can be used to develop mitigation strategies, and HBM can thus support the central 'Leaving no one behind' target of the 2030 UN agenda for Sustainable Development (Ganzleben and Kazmierczak, 2020; UN, 2015).

Although there are some good examples on the successful use of HBM in regulatory RA both from the EU (Louro et al., 2019) and e.g. from North America (Gurusankar et al., 2017; Health Canada, 2016a, 2016b; Zidek et al., 2017), there is still room for improvement on the use of HBM in RA. As part of the HBM4EU, we have previously published the results of a survey on national regulatory risk assessors on their day-to-day RA practices and the use of HBM data (Louro et al., 2019). The survey showed a general lack of knowledge on and experience in the use of HBM data among the European regulatory risk assessors, and a need for guidance on how to integrate HBM in different RA procedures (e.g., under REACH, the cosmetics regulation or the biocidal products regulation). In the survey, 64% of the respondents indicated that hardly any guidance was available in their country on the use of HBM in general population RA, and, in the cases in which some guidance was available, it was usually specific to a single regulatory domain (Louro et al., 2019). For example, the human health RA scheme for biocidal active substances does not include specific guidance on the use of HBM, but the guidance for human health RA makes some references to it (ECHA, 2017b). Another key finding by (Louro et al., 2019) was that scientifically sound, health-based biological guidance or limit values should be developed to ensure better use of HBM data in RA, preferably prepared at the EU and/or global (e.g., WHO, FAO) level, and have at least some regulatory recognition.

These results support earlier findings that guidance on how to use HBM in risk characterisation and management is limited (Boogaard et al., 2011; ECHA, 2012). Surprisingly, this is the case for both the general and the occupational population, despite the long-standing tradition of HBM in occupational health. In the occupational field, concerns regarding the ethical aspects related to HBM were raised in the survey (Louro et al., 2019). This issue has also been discussed recently in a paper by (Viegas et al., 2020), who emphasise the need for guidance to clarify several aspects, including the role of HBM in workplace exposure and RA, risk management versus workers' health surveillance, HBM campaign management, and how to deal with ethics and the European General Data Protection Regulation (GDPR). Recently, OECD has published a guidance for setting up and using biological limit values for the internal exposure of workers (OECD, 2022). Some earlier examples and guidance on the application of HBM in general population RA have been provided by (Gurusankar et al., 2017). At that time, only a handful of examples existed for incorporating HBM data into health RA. This paper aims to provide further examples and guidance on the use of HBM in RA and its application to different regulatory schemes, based on work from the HBM4EU initiative. The major goal is more effective support of the integration of HBM into regulatory RA, with a focus on European RA practises. We concentrate on exposure assessment through HBM, but also offer some insights into the use of effect biomarkers. We elaborate on many HBM-related aspects not previously described in detail in the relevant RA guidance documents, a list of which can be found in Supplementary Table 1.

The regulatory frameworks and processes considered here include EU REACH, biocidal products, plant protection products (PPP), occupational safety and health (OSH), and cosmetic product regulations. However, HBM also has broader applications in chemical monitoring and management outside of these and other regulatory contexts, including post-marketing surveillance, the identification of emerging concerns, hotspot identification and monitoring, awareness raising, and monitoring of the effectiveness of risk management measures (Colles et al., 2019; Kolossa-Gehring et al., 2012; Reynders et al., 2017).

It should be noted that the RAs and human health impact assessments (made by calculating environmental burden of disease (EBoD)) in this paper are not intended to have direct regulatory implications. They represent exemplary RAs and illustrate how HBM can be used in RA and

Table 1
Summaries of methodological approaches used in RA and EBoD estimations.

Substance Group, Specific substances included in RA/EBoD	Population covered	Exposure assessment	Key endpoint	Biological value for internal exposure/Point of departure (PoD)/Existing dose–response used	Approach for reverse/forward calculation, if applicable	RA/EBoD methodology	Reference for full RA or EBoD estimations
Acrylamide	General (children and adults)	Data from HBM4EU-aligned studies (aggregated data), based on acrylamide urinary metabolite AAMA	Cancer, peripheral neuropathy	Cancer: A BMDL10 value (0.17 mg/kg bw/d) for neoplastic effects in mice (EFSA, 2015b) was converted into the human equivalent, and linear extrapolation was performed resulting in extra cancer risk of 2.4×10^{-3} per µg/kg bw/d. Peripheral neuropathy: a provisional HBM-GVs (0.32 and 0.29 mg AAMA/L urine) was derived on the basis of the BMDL10 value (0.43 mg/kg bw/d) for non-neoplastic effects in rats, derived by (EFSA, 2015b).	Measured AAMA levels were converted into external intakes using the urinary mass-balance approach (Apel et al., 2020) with a fractional urinary excretion (F_{UE}) used earlier by (Hays and Aylward, 2008).	Cancer: Linear extrapolation was used to estimate cancer risks at specific intake levels. P95 levels of urinary AAMA levels, reverse calculated as external intake levels, were used as reasonable worst-case exposure estimates for risk characterisation. Peripheral neuropathy: Direct comparison of measured AAMA levels (P95), reverse calculated as external intake levels, with the provisional HBGV to calculate RCRs[a].	(Govarts et al., 2023; Mahiout et al., 2022b)
Anilines: ortho-toluidine	General (adults), Workers (adults)	Published data from literature (aggregated data), urinary o-toluidine including hydrolysed metabolites	Cancer	Dose–response for carcinogenicity as derived by SCOEL (European Commission et al., 2017b) based on BMDL10 (42.2 mg/kg bw/d) for neoplastic effects in rats was used for workers resulting in extra cancer risk of 0.48×10^{-3} per mg OT/m^3. For general population extra cancer risk was 9.4×10^{-3} per mg OT/kg bw/d (Huuskonen et al., 2022).	Urinary mass-balance approach (Apel et al., 2020) with a F_{UE} of 0.75 and PBPK modelling was used to convert biomonitoring data into external intake.	Cancer: Linear extrapolation was used to estimate cancer risks. Mean and P95 levels of the urinary o-toluidine levels, reverse calculated as external intake levels, were used as reasonable exposure estimates for risk characterisation.	Huuskonen et al. (2022)
Aprotic solvents: NMP (1-methyl-pyrrolidin-2-one), NEP (1-ethylpyrrolidin-2-one),	General (children, adolescents, young adults)	NMP, NEP: Published German HBM data on urinary NMP metabolites 5-HNMP and 2-HMSI, and urinary NEP metabolites 5-HNEP and 2-HESI;	Developmental toxicity (NMP and NEP), liver toxicity (DMF)	NMP and NEP: HBM-GV_{GenPop} of 15 mg/L for adolescents and adults and 10 mg/L for children (David et al., 2021)	nr[b]	Direct comparison of HBM data (GM, median, P95) with HBM-GVs determined under HBM4EU.	Mahiout et al. (2022b)
DMF (N,N-dimethyl-formamide)		DMF: German HBM data on urinary metabolite AMCC.		DMF: HBM-$GV_{workers}$ of 10 mg/g creatinine (Lamkarkach et al., 2022) was divided by an assessment factor of 10 to consider more sensitive population subgroups, yielding a provisional HBM-GV_{GenPop} of 1 mg/g creatinine for DMF metabolite AMCC (Mahiout et al., 2022b).			
Arsenic: inorganic arsenic (iAs), monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA)	General (infants, children, adolescents, adults)	Published data from literature and HBM4EU-aligned studies (aggregated data)	Lung, bladder, and skin cancer	BMDL 0.5 = 3.0 µg/kg bw/d defined by (WHO/FAO, 2011) was used as a PoD for linear extrapolation as explained by ECHA/RAC (ECHA, 2013b, 2017a). This resulted in a cancer slope of 1.7/1000 for 1 µg iAs/kg bw per day.	The sum of iAS, MMA and DMA levels was converted into external intakes using the urinary mass-balance approach (Apel et al., 2020) with an F_{UE} used earlier by (Hays et al., 2010b).	Cancer risk was estimated on the basis of the urinary excretion of iAs, MMA and DMA, converted into external intake levels. Cancer risk was calculated using the cancer slope of 1.7/1000 for 1 µg iAs/kg bw per day.	Mahiout et al. (2022b)

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Table 1 (*continued*)

Substance Group, Specific substances included in RA/EBoD	Population covered	Exposure assessment	Key endpoint	Biological value for internal exposure/Point of departure (PoD)/Existing dose–response used	Approach for reverse/forward calculation, if applicable	RA/EBoD methodology	Reference for full RA or EBoD estimations
						Inorganic As intake calculated from food consumption was compared to retrieved data on urinary excretion of iAs, MMA and DMA.	
	General	Published data from literature and HBM4EU-aligned studies (aggregated data) of adolescents	Hyperpigmentation and vascular complications	Biomonitoring equivalent (BE) value of 6.4 μg/L for toxicologically relevant arsenic (As(III)+As(V)+MMA + DMA) (Hays et al., 2010a)	nr	Direct comparison of P50 and P95 levels with BE value.	Buekers et al. (2023)
Bisphenols: bisphenol A (BPA), bisphenol S (BPS)	General (adults)	Published data from literature and HBM4EU-aligned studies (aggregated data)	BPA: effect on renal mean relative weight in animals (EFSA's t-TDI (2015c) or adverse effects on immune system in animals (EFSA's new TDI proposal 2021 ([7] below) BPS: effect on mammary gland and neurobehaviour of F1 in animals	Urinary total HBM-GV_{GenPop} of 230 μg/L and 1.0 μg/L were used for BPA and BPS, respectively (Ougier et al., 2021c)	nr	P95 reported in the aligned studies were compared to the corresponding HBM-GV_{GenPop} to calculate the corresponding RCRs.	(Mahiout et al., 2022b; Meslin et al., 2022)
Cadmium	General (elderly women)	DEMOCOPHES data and published data from literature	Osteoporosis in elderly women	(Engstrom et al., 2011) threshold: 0.5 μg Cd/g creatinine Odds ratio: 1.61 (1.20–2.16) for 0.50–0.75 μg U–Cd/g creatinine and 1.95 (1.30–2.93) for ≥0.75 μg U–Cd/g creatinine	nr	EBoD calculation of osteoporosis associated with Cd exposure; Methodology followed comparative RA approach. Attributable fraction was calculated, and costs (direct, indirect, intangible) estimated.	Ougier et al. (2021a)
	General (adults)	HBM4EU-aligned studies (aggregated data)	Chronic kidney disease	Age-specific HBM-GVs for Cd: 0.2 μg Cd/g creatinine for 11–20 years, 0.3 μg Cd/g creatinine for 21–30 years and 0.5 μg cd/g creatinine for 31–40 years old adults (Lamkarkach et al., 2021)	nr	Direct comparison of age-specific HBM-GVs for Cd with urinary Cd concentrations (P95) from adults in the HBM4EU-aligned studies (20–39y). Percentage of individuals exceeding HBM-GVs was calculated.	Snoj Tratnik et al. (2022)
Chromium: hexavalent chromium [Cr(VI)]	Workers (adults)	Finnish Institute of Occupational Health's (FIOH) HBM database, (aggregated data) on urinary total chromium (U–Cr) during 1980–2016	Lung cancer	Dose–response for lung cancer relative risk (RR) by (Seidler et al., 2013), based on a meta-analysis of epidemiological studies. In addition, excess life-time cancer cases estimated using the cancer risk evaluations of ECHA (2013a), DECOS (2016) and SCOEL (European Commission et al., 2017a)(4×10^{-3} per μg Cr (VI)/m^3).	U–Cr data were converted into corresponding Cr(VI) air levels using published regression formulae (Chen et al., 2002; Lindberg and Vesterberg, 1983; Viegas et al., 2022) to enable lung cancer RA.	Lifelong occupational exposure to Cr(VI) was covered (40 years during 1980–2019). As the lung-cancer dose–response was based on external Cr(VI) exposure, published regression formulas were used to relate internal U–Cr levels (P95) with external inhalation exposure estimates. This enabled subsequent RA on the basis of the published lung cancer RR dose–response and the estimates of excess cancer cases.	Mahiout et al. (2022a)

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Table 1 (*continued*)

Substance Group, Specific substances included in RA/EBoD	Population covered	Exposure assessment	Key endpoint	Biological value for internal exposure/Point of departure (PoD)/Existing dose–response used	Approach for reverse/forward calculation, if applicable	RA/EBoD methodology	Reference for full RA or EBoD estimations
Diisocyanates: MDI (4,4′-methylene diphenyl diisocyanate), 2,4/2,6-TDI (toluene diisocyanate), HDI (hexa-methylene-diisocyanate)	Workers (adults)	FIOH's HBM database for metabolites MDA, TDA, HDA.	Bronchial hypersensitiveness (BHR)	RAC dose–response (e.g. 0.12–0.19 μg/m^3 NCO causes 2% BHR risk) (ECHA, 2020) for working life long excess BHR risk was used for the risk assessment.	External exposures to MDI and 2,4/2,6-TDI were reconstructed by a developed PBPK method (Scholten et al., submitted), and HDI by a published correlation equation (Maitre et al., 1996).	BHR risk was assessed on the basis of the estimated distributions of external NCO air concentration, reconstructed from urinary diamine levels using the RAC dose–response, or the published correlation equation. Due to the highly skewed distribution with few very high exposures driving the higher percentages, estimated excess risks beyond 7.5% were capped at this level to avoid possible unrealistic extrapolation beyond the highest excess risk reported by RAC.	Huuskonen et al. (2023)
Flame retardants: tris (2-chloroethyl) phosphate (TCEP), tris (chloropropyl) phosphate (TCIPP), tris (1,3-dichloro-2-propyl) phosphate (TDCIPP)	General (children)	HBM4EU-aligned studies (aggregated data) on urinary metabolites BCEP, BCIPP and BDCIPP.	Kidney toxicity	Several health-based guidance values (HBGVs) for external exposure used as minimal risk level and reference doses: 0.2 mg/kg bw/d for TCEP and TDCIPP (ATSDR, 2012), p-RfD of 0.007mg/kg bw/d for TCEP (US-EPA, 2009), RfD of 80 μg/kg bw/d for TCIPP, 22 μg/kg bw/d for TCEP and 15 μg/kg bw/d for TDCIPP (Ali et al., 2012).	HBM data was used to estimate the daily intake of TCEP, TCIPP using means of a urinary mass-balance approach (Apel et al., 2020) with a F_{UE} of 0.63.	Arithmetic mean and P95 levels of HBM data were used to estimate average and high dietary exposure. The occurrence data of selected food categories originated from a Belgium food basket study (Poma et al., 2018) and dietary intake was estimated on the basis of national food consumption data and occurrence data. Both the estimated dietary intake and the estimated daily intake, which was based on HBM data, were compared to the HBGVs and the RCR was calculated.	Plichta et al. (2022)
Lead	General (infants, children, adolescents, adults)	Public literature data and Slovenian HBM data on blood lead levels (BLL)	Children/adolescents: develop-mental neurotoxicity (lost cognitive development)	Children: dose–response functions for neurodevelopmental effects; −0.054 (95% CI: -0.034 to −0.075) for BLL between 20 and 100 μg/L; −0.019 (95% CI: -0.012 to −0.026) for BLL between 100 and 200 μg/L; −0.011 (95% CI: -0.007 to −0.015) for BLL between 200 and 300 μg/L (Lanphear et al., 2005; Remy et al., 2019)	nr	EBoD was estimated in terms of disability-adjusted life years (DALYs, per 100,000) due to exposure to lead (Pb). Children/adolescents: DALYs based on a log-linear relationship between BLL and total numbers of Full–Scale IQ points (FSIQ) loss attributable to BLL above 20 μg/L. Sensitivity analysis (considering no threshold in Pb exposure) included all ranges of BLL.	Mahiout et al. (2022b)
			Adults: premature mortality (all- cause mortality ICD-10, chapters I –XVIII and XX[c]; adults aged over 20)	Adults: Dose–response relationship (hazard ratio) for all-cause mortality; 1.37 (95%CI: 1.17–1.60) (Lanphear et al., 2018)		Adults: DALYs using dose–response relationship and corresponding hazard ratios (HR) for BLL above 10	

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Table 1 (*continued*)

Substance Group, Specific substances included in RA/EBoD	Population covered	Exposure assessment	Key endpoint	Biological value for internal exposure/Point of departure (PoD)/Existing dose–response used	Approach for reverse/forward calculation, if applicable	RA/EBoD methodology	Reference for full RA or EBoD estimations
						µg/L. In the SLO case, DALYs were also presented as a mean and 95% confidence interval.	
Mercury: methylmercury	General (children and women of childbearing age)	Public literature data on blood and hair mercury levels	Neurotoxicity	HQ[d] approach: HBM-GV 5 µg/L (HBM-I) in blood by the German HBM Commission (Schulz et al., 2007) EFSA approach: TWI of 1.3 µg/kg bw established by [(EFSA, 2012); equivalent to a TDI of 0.19 µg/kg bw/d, which corresponds to hair levels of 1.9 µg/g]	EFSA TWI/TDI was converted into hair levels using earlier published correlations (WHO, 2008). The published ratio was also used for hair-Hg to blood-Hg conversion (Esteban-Lopez et al., 2022; FAO/WHO, 2004; WHO, 1990)	HQ approach: HQ were calculated as the ratio of the biomarker concentration (as mercury in blood, GM, P95) to the HBM-GV (HBM-I) for mercury. EFSA approach: Exposure levels (mercury in hair, GM, P95) compared to tolerable weekly intake levels.	(Dominguez-Morueco et al., 2022; Mahiout et al., 2022b)
Mycotoxins: deoxynivalenol and its derivatives (total DON)	General (children, adolescents, adults)	Public literature data and HBM4EU-aligned studies (aggregated data) on total urinary DON	Reduced body weight gain in experimental animals	HBGV: TDI 1 µg/kg bw/day HBM-GV 23 µg/L for urinary total DON (Apel et al., 2022)	Probable daily intakes were reverse-calculated from DON and its metabolite levels using the urinary mass-balance approach (Apel et al., 2020) with an F_{UE} of 0.64.	Two approaches: I) hazard quotient calculated by comparing estimated probable daily intakes (mean, median, maximum) to TDI II) direct comparison of HBM data (mean, median, maximum levels) with HBM-GV	(Alvito et al., 2022; Mahiout et al., 2022b)
PAHs: 1-hydroxy-pyrene (1-OHPyr) as surrogate for PAH4 (BaA, BbF, BaP, CHR)	General (adults)	HBM4EU-aligned studies (aggregated data) on urinary 1-OHPyr	Cancer	RAC ECHA (ECHA, 2018) excess lifetime cancer risk (ELCR) dose–response relationship, using BMDL10 (=340 µg/kg bw/day) for PAH4 derived from animal studies on coal tar (EFSA, 2008) which gives, in combination with an allometric scaling factor of 7, an ELCR unit of $2.06 \times 10^{-3} \times$ 'exposure dose' per µg/kg bw/day, where 'exposure dose' refers to the median dietary (oral) intake of PAH4.	Probable Daily Intake, (PDI) of pyrene was reverse-calculated from HBM aligned studies data on 1-OHPyr, using PBPK INTEGRA modelling (Sarigiannis et al., 2020).	Following approach was used: 1. Pyrene intake (PDI) was estimated from HBM data on 1-OHPyr, 2. Pyrene intake was then associated with PAH4 intake, based on the assumption that pyrene was a surrogate of PAH4. 3. PAH4 intake was alternatively derived from the country specific food residue and the food consumption data, available in the EFSA reports (EFSA, 2008, 2015a). 4. ELCR was calculated for both sets of PAH4 intake data using the ECHA-RAC dose–response relationship (ECHA, 2018) for PAH4.	Mahiout et al. (2022b)
Pesticides: chlorpyriphos and chlorpyriphos-methyl	General (adults and children)	HM4EU-aligned studies (aggregated data) based on U-TCPy levels	Develop-mental neurotoxicity, complemented with specific (AChE) and generic (chronic toxicity and carcinogenicity) endpoints.	Different endpoints covered: HBM-PoD[e] 5.94 (adults) and 3.96 (children) mg/L based on the urinary marker 3,5,6-trichloro-2-pyridinol (TCPy) for overall developmental neurotoxicity; 1.98 (adults) and 1.32 (children) mg/L for long-term and maternal no-observed-adverse-effect level (NOAEL), and for short-term NOAEL for red blood cells AChE; and 198.1 (adults) and 137.1	Urinary mass-balance approach was used to convert external PoDs into internal PoDs (Apel et al., 2020). F_{UE} used for forward calculation was 0.7.	The margin of exposure (MoE) approach was used for RA. The level of the concerns associated with the MoE were assessed on the basis of the standard uncertainty factors used for each endpoint and the confidence in the PoD value. Risk levels for the MoE results were graded using colour codes to facilitate risk communication:	Tarazona et al. (2022b)

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Substance Group, Specific substances included in RA/EBoD	Population covered	Exposure assessment	Key endpoint	Biological value for internal exposure/Point of departure (PoD)/Existing dose–response used	Approach for reverse/forward calculation, if applicable	RA/EBoD methodology	Reference for full RA or EBoD estimations
				(children) for carcinogenicity NOAEL.		Green: low risk, MoE at least 10 times higher than conservative standard factors. Yellow: concerns cannot be ignored, MoE less than 10 times higher than conservative standard factors. Orange: possible concerns, MoE lower than conservative standard factors. Red: confirmed concerns, MoE lower than minimum standard factors.	
Pesticides: pyrethroid mixture: bifenthrin, cyfluthrin, cypermethrin, deltamethrin, etofenprox, fenpropathrin, fenvalerate, λ-cyhalothrin, permethrin, tau-fluvalinate	General population (adults and children)	HM4EU-aligned studies (aggregated data), based on urinary levels of -ClF3CA, trans-CDCA, 3-PBA, 4-FPBA, cis DCCA, trans DCCA, DBCA	Neurotoxicity/ developmental neurotoxicity.	Metabolite 3-PBA was used as a general biomarker for pyrethroid exposure: derivation of screening values for children and adults under worst-case assumptions adults 4.8 μg 3-PBA/L; children 3.25 μg 3-PBA/L (based on information obtained with different parent compounds), with several additional refinements including a probabilistic distribution of HBM screening values. Cyfluthrin: adults 130 μg 4-FPBA/L, children 80 μg 4-FPBA/L; deltamethrin: adults 130 μg DBCA/L, children 90 μg DBCA/L urine; corresponding to the HBM-GVs for general population previously proposed (Apel et al., 2022). For other pyrethroids: bifenthrin (adults 90 μg CIF3CA/L; children 60 μg CIF3CA/L), lambda-cyhalothrin (adults 14 μg CIF3CA/L; children 9 μg CIF3CA/L), cypermethrin (adults 45 μg DCCA/L; children 30 μg DCCA/L), and permethrin (adults 0.48 mg DCCA/L; children 0.32 DCCA/L); provisional HBM-GVs were derived on the basis of ADIs established by either EFSA or WHO (JMPR)	Provisional HBM-GVs were derived from existing ADIs using the urinary mass-balance approach for forward calculation. (Apel et al., 2020). F_{UE} for individual pyrethroids varied between 0.09 and 0.85	The tiered approach was used. Aggregated HBM data (median and P95 and their confidence intervals) obtained from HBM4EU-aligned studies were used as conservative estimates of population exposure levels in the first step. In the refined assessment, the probabilistic refinement using the full distributions of the metabolite levels were used for Monte Carlo analysis.	Tarazona et al. (2022a)
PFAS mixtures: PFBS, PFHxS, PFHpS, PFOS, PFHxA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA	General (adolescents)	HBM4EU-aligned studies (individual data), based on plasma/serum concentrations	Immunotoxicity, birth weight reduction	HI approach, PoD: human internal exposures associated with a given effect on immunotoxicity or birth weight reduction. RPF and EFSA sum value approach, HBM-GV: EFSA TWI, based on a $BMDL_{10}$ for immune	nr	Mixture risk assessment (MRA) was conducted on the basis of the Relative Potency Factor (RPF) approach, the Hazard Index (HI) approach, and the sum value approach	Bil et al. (2023)

(*continued on next page*)

Table 1 (*continued*)

Substance Group, Specific substances included in RA/EBoD	Population covered	Exposure assessment	Key endpoint	Biological value for internal exposure/Point of departure (PoD)/Existing dose–response used	Approach for reverse/forward calculation, if applicable	RA/EBoD methodology	Reference for full RA or EBoD estimations
				suppression in children (17.5 ng/mL), interpreted at blood serum level in their mothers (6.9 ng/mL) (EFSA, 2020).		of the European Food Safety Authority (EFSA).	
Phthalates mixture: DEHP, DiBP, DnBP, BBzP and DiNP	General (children and adolescents)	HBM4EU-aligned studies (individual data), based on urinary metabolite concentrations	Anti-androgenic effects (e.g., foetal testicular testosterone suppression, germ cell depletion, testicular changes)	HBM-GVs were used for BBzP, DEHP, DiBP and DnBP (Lange et al., 2021). For the adult general population incl. adolescents, the HBM-GV_{GenPop} for the specific metabolite(s) of the respective parent compounds in urine are the following: 0.5 mg/L for the sum of 5-oxo-MEHP and 5-OH-MEHP; 0.19 mg/L for MnBP, 0.23 mg/L for MiBP; 3 mg/L for MBzP. For children, the HBM- GV_{GenPop} for the specific metabolite(s) of the respective parent compounds in urine are the following: 0.34 mg/L for the sum of 5-oxo-MEHP and 5-OH-MEHP; 0.12 mg/L for MnBP, 0.16 mg/L for MiBP; 2 mg/L for MBzP. For DiNP, provisional HBM-GV_{GenPop} (sum of cx-& OMiNP 0.34 and 0.51 mg/l for children and adults, respectively) was derived solely for the purpose of a mixture risk assessment based on foetal testis testosterone suppression observed in animal studies (Lange et al., 2022)	Provisional HBM-GV_{GenPop} for DINP was derived from external PODs using the urinary mass-balance approach (Apel et al., 2020) as had been applied earlier for other phthalates (Lange et al., 2021).	Hazard index (HI) approach using HBM-GVs; Comparison of individual exposure levels with HBM-GV_{GenPop} for children (<14 years) and adolescents (>14 years) to obtain the risk quotients (RQ) of each of the five phthalates; RQs were summed to obtain individual HI; For comparison, a precautionary factor of 5 and 10 was applied, yielding adapted HI values of 0.1 and 0.2, respectively, to account for other anti-androgenic substances not assessed. Individual HI were compared to risk thresholds of HI > 1, 0.2, 0.1.	Lange et al. (2022)
UV filters: benzophenone-3 (BP-3)	General (adolescents, adults)	HBM4EU-aligned studies (aggregated data), creatinine-corrected urine concentrations	Reduction in spermatocytes	A provisional HBM-GV of 340 μg/g creatinine was derived on the basis of the reduction in the number of spermatocytes per seminiferous tubule in offspring. The POD was the NOAEL of 67.9 mg/kg bw/day from the most recent SCCS opinion (SCCS, 2021).	The urinary mass-balance approach (Apel et al., 2020) was applied to convert the external POD into a reference urinary excretion level of benzophenone-3. F_{UE} used for forward calculation was 0.01.	The P50 and P95 of the BP-3 measurements were compared with the provisional HBM-GV, using the RCR approach.	(Mahiout et al., 2022b; Rousselle et al., 2022)

[a] RCR = Risk characterisation ratio, used interchangeably with HQ=Hazard quotient.
[b] nr = Not relevant.
[c] ICD= International Statistical Classification of Diseases and Related Health Problems by WHO, 10th Revision: https://icd.who.int/browse10/2019/en.
[d] HQ=Hazard quotient, used interchangeably with RCR = Risk characterisation ratio.
[e] PoD=Point of Departure.

EBoD estimations. However, as newly generated HBM data have been used in many of them, the results can also be useful for raising awareness of possibly needed policy measures.

The abbreviations used have been listed in the supplementary material attached to the paper.

2. Methodological approach

One of the objectives of the HBM4EU was to explore how HBM data can be used in chemical RA or EBoD calculations, and to demonstrate the benefits and challenges. The focus was on the HBM4EU priority substances/substance groups (Ougier et al., 2021b): acrylamide, the aniline family, aprotic solvents, arsenic, benzophenone UV-filters, bisphenols, cadmium, chemical mixtures, hexavalent chromium [(Cr(VI)], diisocyanates, emerging chemicals, flame retardants, lead, mercury, mycotoxins, per-/poly-fluorinated compounds, pesticides, phthalates and DINCH, and polycyclic aromatic hydrocarbons (PAHs).

To demonstrate how HBM data can be effectively used in RA and how the critical aspects related to the use of HBM data can be identified, we conducted RAs of the priority substances using HBM data. We also made EBoD calculations for a selection of these substances, exploring the burden of disease related to environmental chemical risk factors. EBoD calculations were made following the principles described in (Hänninen et al., 2014).

The general approach consisted of the following steps.

- Identifying existing RAs for the substance
- Identifying the existing health-based guidance or limit values for internal exposure (examples provided in Supplementary Table 2), including human biomonitoring guidance values (HBM-GVs) from the HBM4EU project (Apel et al., 2020), HBM values from the German HBM Commission, biomonitoring equivalents (BEs) from the consulting firm Summit Toxicology and Health Canada, and biological limit values (BLVs) in the occupational area. Of these, we gave preference to the HBM-GVs, if they were available. If internal guidance or limit values were not available, we identified the existing health-based guidance or limit values for external exposure (e.g., ADIs, TDIs, TWIs, BMDLs).
- If no health-based guidance or limit values for internal exposure existed, we identified suitable approaches for estimating external intake from biomarker levels, or for converting health-based guidance or limit values for external exposure as biomarker levels (reverse or forward calculation, respectively). These included the use of physiologically based pharmacokinetic (PBPK) modelling, the urinary mass-balance approach based on one-compartment modelling (Apel et al., 2020; Hays and Aylward, 2009; Hays et al., 2008) or the use of measured correlations (regressions) between external exposure and internal levels. These approaches were subsequently used to calculate the biomarker levels corresponding to the health-based values for external intake
- In some cases, for example, lead and cadmium, we used published effect estimates from epidemiological studies on the associations between biomarker levels and health effects for RA/EBoD estimations
- Identifying relevant HBM data to be used for the RA/EBoD calculations
- Performing RA or EBoD calculations based on HBM data.
- Analysing the benefits and challenges, including uncertainties, of using HBM in RA in comparison to using external exposure data

The HBM data for the RAs and EBoD calculations were acquired from either the published literature or from HBM4EU-aligned studies. In some cases, data from the participating institutions' own databases were also included. The HBM4EU-aligned studies were surveys that collected HBM samples and data in as harmonised a way as possible from (national) studies, in order to derive current internal exposure data from the European population across a geographic spread (Gilles et al., 2021, 2022). These data were further harmonised, transformed, and statistically analysed (Gilles et al., 2022; Govarts et al., 2023)). Unless otherwise stated, we used summary statistics (aggregated data) for the assessments.

We next give an overview of the assessments conducted. The methodological approach used in each assessment is briefly described in Table 1. The detailed, full RAs or EBoD calculations have been published in scientific journals or in HBM4EU deliverable reports and are available in the references provided in Table 1.

3. Results

The summarised results of the RAs and EBoD calculations are presented below. References for the full RAs or EBoD calculations are also provided.

3.1. Acrylamide

The cancer RA was based on the acrylamide urinary metabolite (AAMA) levels measured in the HBM4EU-aligned studies (Mahiout et al., 2022b). The measured geometric mean (GM) and P95 levels of AAMA (20–100 μg/L and 70–510 μg/l, respectively) in adults and children were converted into external exposure estimates using the urinary mass-balance approach and the cancer risk was calculated by assuming a linear dose–response. The estimated cancer risks were 1:100–1:1000, which is in accordance with the earlier RA of the EFSA (2015b), which was based on external intake estimates. For the assessment of the peripheral neuropathy risk of acrylamide, provisional HBM-GV was derived using the (EFSA, 2015b) BMDL10 of 0.43 mg/kg bw/d as the point of departure, and the urinary mass-balance approach to convert external intake levels into biomarker levels. The mean acrylamide levels were below the provisional HBM-GVs 0.32 and 0.29 mg AAMA/l urine in children and adults, respectively. However, in two studies, 95th percentile of acrylamide levels in adults exceeded the provisional HBM-GV, giving risk characterisation ratio (RCR) values of 1.05 and 1.75, which indicates an increased risk of peripheral neuropathy (Govarts et al., 2023). The main uncertainties in the RA were related to the cancer dose–response and the linear extrapolation of cancer risk based on animal data. In addition, the endogenous production of AAMA may overestimate exposure up to two-fold. However, considering that the results of the cancer RA were very close to the results of the EFSA RA, this specific uncertainty in this biomonitoring approach is considered rather minor.

3.2. Anilines

From the chemical group of anilines, cancer RA was performed for ortho-toluidine (o-toluidine) on the basis of the published literature data on the general population and workers (Huuskonen et al., 2022). The reverse calculation of urinary o-toluidine levels used both the urinary mass-balance approach and a general PBPK model created for the INTEGRA platform. The external exposure estimates derived by using different approaches showed about a 30% difference, which can be explained by the PBPK model considering exposure dynamics that capture the intra-day variability of urinary o-toluidine, whereas the urinary mass-balance method is based on steady-state urinary levels. However, the 30% difference is rather minor considering the uncertainties related to, for example, the cancer dose-response based on animal data. It was estimated that workers exposed to o-toluidine are at a 6 to $9{:}10^5$ risk of cancer in the worst-case scenario (0.9 mg/L of o-toluidine in urine, P95 level). In the general population, the exposure levels and cancer risk of o-toluidine were orders of magnitude lower than those of the workers. The main uncertainty in this RA was related to the limited HBM data on both the general population in Europe and workers, as o-toluidine was not included in the HBM4EU-aligned studies.

3.3. Aprotic solvents

In the RA (David et al., 2021), HBM-GVs$_{GenPop}$ were used for NMP and NEP (David et al., 2021), whereas a provisional HBM-GV$_{GenPop}$, derived from the HBM-GV$_{worker}$ value (Lamkarkach et al., 2022), was used for DMF. NMP and NEP exposure data were obtained from two studies conducted in Germany, which used data on adults from the German Environmental Specimen Bank (ESB), taken between 1991 and 2014 (Ulrich et al., 2018), and data from the German Environmental Survey of Children and Adolescents V (GerES V) (Schmied-Tobies et al., 2021). In addition, DMF samples from the ESB were analysed for the metabolite AMCC for the period 2000 to 2021 (data unpublished). The exposure of adults (Ulrich et al., 2018), children and adolescents (Schmied-Tobies et al., 2021) was well below the guidance values for both NMP and NEP. The maximum values of the studies were 4.7–10 factors lower than the corresponding HBM-GV$_{GenPop}$ values. The maximum value found in the data from ESB on the DMF metabolite AMCC was a factor of 2.5 lower than the provisional HBM-GV$_{GenPop}$ of 1 mg/g creatinine.

Even when the combined exposure to NMP and NEP was considered, the values were not exceeded. The calculated hazard index (HI) was well below 1 in all the cases considered (i.e., children, adolescents, and adults) with maximum HI values of 0.3. The HI for young adults was calculated using the combined exposure to NMP, NEP and DMF, resulting in a maximum HI value of 0.6. However, in 'real-life situations', possible combined exposure with other reprotoxic substances present in the environment should be considered, as these might increase the risk of common effects. The main uncertainty in this RA was the fact that the exposure data were only available from Germany.

3.4. Arsenic

The urinary mass-balance approach was also used in the case of arsenic to convert external intake of iAs into the sum of urinary iAs, MMA and DMA (Mahiout et al., 2022b). The HBM data from the literature and the HBM4EU-aligned studies showed that in most studies, the 95th percentile levels of the general population exceeded the biomonitoring equivalent (BE) value of 6.4 μg/L derived by (Hays et al., 2010a). In some studies, even median exposure levels exceeded the BE. The BE value is based on non-cancer effects (hyperpigmentation and vascular complications) with a point of departure (PoD) of 0.8 μg/kg bw/d, based on a human study (Hays et al., 2010a). Assuming steady state conditions, this corresponds to U–As levels of 19.3 μg/L. By applying an assessment factor of 3 for interindividual differences, a value of 6.4 μg/L was obtained.

The existence of a threshold value for carcinogenic effects on the lungs and bladder has been discussed extensively (Tsuji et al., 2021). Based on reversed dosimetry and HBM data, an average dose of 0.16 μg kg^{-1}bw/day was estimated for inorganic arsenic (As(III) +As(V)) exposure. Linear extrapolation, based on a lifetime excess lung cancer risk of 1.7×10^{-3} per 1 kg^{-1} bw/day (ECHA, 2013b), gives a cancer risk of 2.7×10^{-4}. Although this is in line with the RA of EFSA, which was based on estimated food intake (EFSA, 2021), it must be interpreted with caution, given the discussion on the possibility of a threshold value. In addition to the dose–response, another important uncertainty is related to the overestimation of iAs exposure due to the widespread presence of DMA in food. Analytical challenges related to the speciation of As species, and the representativeness of the populations studied, can also be considered uncertainties.

3.5. Bisphenols

An RA was performed for bisphenol A and S, for which sufficient HBM data were available and for which HBM-GVs were derived (Mahiout et al., 2022b; Meslin et al., 2022). Bisphenol F was also addressed, but no HBM-GV could be derived, and thus, no RA was conducted for this substance.

The HBM data were gathered from the HBM4EU data repository, and a literature search was performed. In comparison to the HBM-GVs, the calculated RCRs were very low for Bisphenol A (BPA), ranging from 0.01 to 0.14 (all populations combined), contrary to those obtained for Bisphenol S (BPS) ranging from 0.4 to 28.9 (all populations combined). This was due to the fact that the HBM-GVs derived for BPS are based on endocrine-disrupting health effects occurring in animals at very low doses, contrary to the values calculated for BPA on the basis of the temporary tolerable daily intake (t-TDI) from EFSA (EFSA, 2015c). A comparison with the revised tolerable daily intake (TDI), recently opened for comments by the EFSA,[1] clearly show that risks for the whole population cannot be ruled out. If this new TDI is confirmed, comparing the P95 for total BPA measured in the aligned studies to the HBM-GV derived from the new proposed TDI (2.3 ng total BPA/L urine for adults and 1.4 ng total BPA/L urine for children) would result in RCRs far exceeding 1. Such low-level HBM-GVs are also below the detection limits of most existing analytical methods, meaning that the sensitivity of detection needs to be further improved.

In the occupational field, the assessment of available HBM data indicated that the risk of occupational exposure to BPA and BPS should not be disregarded (Bousoumah et al., 2021; Meslin et al., 2022). A large, potentially exposed occupational group is cashiers. Although the total urinary bisphenol of cashiers was at the same level as that of the general population, their exposure to free (active) bisphenol A via the dermal route was higher than that in the general population oral exposure, which is a concern. Current biomonitoring approaches (total urinary BPA/BPS) cannot measure the magnitude of cashiers' exposure to free PBA/BPS.

3.6. Cadmium

The low environmental exposure levels of Cd have been associated with adverse effects such as renal toxicity and bone effects. HBM studies conducted in three European countries (Belgium, France and Spain) estimated osteoporosis cases attributable to Cd exposure on the basis of measured urinary Cd levels (Ougier et al., 2021a). The targeted population was women aged over 55, for which dose–response associations between urinary Cd levels and osteoporosis were observable. Around 23% of the cases could possibly be attributed to Cd exposure. In a prospective simulation of lifelong urinary Cd concentrations assuming different intake scenarios, future osteoporosis-attributable cases were calculated based on urinary Cd levels measured in women aged under 55. Between 6% and 34% of studied populations aged under 55 are at risk of osteoporosis. The costs associated with the burden of osteoporosis-related fractures attributable to Cd for each country were assessed, and Cd exposure played a major contributing role to the overall social costs related to osteoporosis.

The HBM4EU-aligned studies compared urinary Cd levels with age-specific HBM-GV for kidney effects (Lamkarkach et al., 2021). According to the results, 0.5%–30% of adults' urinary Cd levels (depending on the country) exceed the HBM-GV (Snoj Tratnik et al., 2022).

3.7. Chromium

Lifelong occupational lung cancer risks due to Cr(VI) exposure in welding and chromium-plating activities (Mahiout et al., 2022a) were estimated on the basis of total U–Cr data (P95, representing realistic worst case) spanning almost 40 years (1980–2016). Published regression formulae, based on concurrent measured U–Cr and air Cr(VI) concentration data in workplaces, were used to relate the measured internal

[1] https://www.foodpackagingforum.org/news/efsa-proposes-to-lower-daily-tolerable-intake-of-bisphenol-a and https://connect.efsa.europa.eu/RM/s/publicconsultation2/a0l1v00000E8BRD/pc0109.

and estimated external exposures (Chen et al., 2002; Lindberg and Vesterberg, 1983; Viegas et al., 2022). This enabled subsequent RA, based on a published lung cancer dose–response formula for external Cr (VI) exposure (Seidler et al., 2013). The lifelong external Cr(VI) exposure estimates for the period 1980–2019 were 1.03 mg/m^3 x year for welders and 0.16–0.32 mg/m^3 x year for platers, averaging for welders at 26 μg/m^3 per year and for platers at 4–8 μg/m^3 per year. Based on these results, realistic worst-case lifelong occupational lung cancer RRs, representing ratios of the probability of lung cancer occurring in the group occupationally exposed to Cr(VI) versus the occupationally non-exposed group, were up to 2.80 for welders and 1.28–1.56 for platers. Attributable risks (ARs), representing the excess risk caused by the exposure, were 64% for welders and 22–36% for platers. When the same calculations were made using the HBM median values, all the RRs were close to 1 and the ARs ≤10% for both platers and welders. Based on the excess cancer risk estimations of the Committee for Risk Assessment (RAC) (ECHA, 2013a), the Dutch Expert Committee on Occupational Safety (DECOS) (2016) and the Scientific Committee on Occupational Exposure Limits (SCOEL) (European Commission et al., 2017a), the estimated worst-case lifelong exposures would correspond with rather high excess lifetime cancer risks (ELCRs) of 98 × 10^{-3} for welders and 16–32 × 10^{-3} for platers, meaning 16–32 and 98 excess lung cancer cases per 1000 workers due to occupational Cr(VI) exposure in plating and welding, respectively.

Several uncertainties may have impacted the RA. The main ones are related to the reliability and applicability of the correlations between U–Cr and air-Cr(VI) levels. Viegas et al. (2022) showed that the U–Cr and air-Cr(VI) correlations among chrome platers were different to those among welders due to different Cr(VI) species, and thus may be partly task specific. Similarly, the RR formula by Seidler et al. (2013) was based on epidemiological data from the chromates industry, and may not be truly representative of welding, as different chromium species are present in these two occupational sectors (Pesch et al., 2018; Scheepers et al., 2008). Welders are also exposed to nickel oxides, which may increase the overall lung cancer risk in this group of workers (Pesch et al., 2019).

3.8. Diisocyanates

Occupational exposure to methylene diphenyl diisocyanate (MDI), toluene diisocyanate (2,4/2,6-TDI), and hexamethylene -diisocyanate (HDI) was assessed on the basis of urinary diamine (U-MDA, U-TDA and U-HDA) data retrieved from FIOH's HBM database (Huuskonen et al., 2023). The database, which is not publicly available, consists of approximately 1000 HBM samples from 2008 to 2021, sent to FIOH for exposure monitoring by occupational health service units. Urinary diamine levels were converted into external isocyanate (NCO) air levels using a PBPK model developed for 2,4/2,6-TDI and MDI (Scholten et al., submitted) and the published correlation between air-HDI levels and U-HDA levels (Maitre et al., 1996). The European Chemicals Agency's (ECHA) RAC exposure-excess risk relation (ECHA, 2020) was used to assess the working life long bronchial hyperresponsiveness (BHR) risk. The entire distribution of air exposures was considered, but excess BHR risks above 7.5% were estimated to result in a 7.5% excess risk to avoid possible unrealistic extrapolations beyond the highest excess risk reported by RAC.

In general, excess risk was the highest for MDI resulting in an excess BHR risk of ≥2.0% in the construction and motor vehicle manufacturing and repair sectors. The assessment included several uncertainties, such as the representativeness of the Finnish biomonitoring data for European workplaces. In comparison to Finnish urinary values, the published data estimated higher exposures in several sectors. The reason why the published literature was not selected was that it did not contain enough information on the distribution of the aggregated exposure levels. Other uncertainties in the RA were related to exposure reconstruction (i.e., lack of validation of the PBPK model and limitations to using a correlation formula) and the lack of information on the number of estimated exposed workers for each sector (affecting the estimated number of expected BHR cases). An advantage of using biomonitoring data for assessing the risk of diisocyanate is that it takes the potential use of respiratory protective equipment and skin exposure into account.

3.9. Flame retardants

HBM data were used to estimate the extent to which the dietary intake of organophosphorus flame retardants (OPFRs) may contribute to the total OPFR exposure among children (Plichta et al., 2022). The urinary mass-balance approach was employed to calculate the estimated daily intake (EDI) of TCEP, TCIPP and TDCIPP. The occurrence data of selected food categories were taken from a published food basket study, and dietary exposure was estimated on the basis of national food consumption data and occurrence data. The results of the HBM4EU-aligned studies showed that the TDCIPP metabolite BDCIPP had the highest detection frequency (37–97.7%). The detection frequency for the TCIPP metabolite BCIPP was below 55% and for the TCEP metabolite BCEP, it ranged between 19 and 63.3% (summarised for Belgium, Denmark, France, Germany, Slovenia, and Slovakia). The estimated daily intakes (EDIs) ranged between 0.03 and 0.18 μg/kg bw/d for TDCIPP, 0.05 and 0.17 μg/kg bw/d for TCIPP, and 0.02 and 0.2 μg/kg bw/d for TCEP. The calculated dietary intakes contributed 11–173% (TDCIPP) and 6–57% (TCEP) to the EDIs. For TCIPP, the estimated dietary intake was above 100% of the EDI, except in the Belgian and French study populations. The 'animal and vegetable fats and oils and primary derivatives thereof', 'grains and grain-based products', 'cheese' and 'milk' food categories were the main contributors to dietary exposure.

For each study population and exposure scenario, the EDI and dietary intake were set according to the available HBGVs derived by (Ali et al., 2012; ATSDR, 2012; US-EPA, 2009). Neither the EDIs nor the estimated dietary intakes of TDCIPP and TCEP exceeded the lowest available HBGVs: RfD of 15 μg/kg bw/d (TDCIPP) and p-RfD of 7 μg/kg bw/d (TCEP). Their maximum contributions to the RfDs were below 1.2% (TDCIPP) and 3% (TCEP). The EDI and dietary intake for TCIPP contributed only 0.01–0.26% to the RfD of 80 μg/kg bw/d. These results suggest that exposure to TDCIPP, TCIPP and TCEP is not likely to cause adverse health effects on the basis of currently available toxicological data. The estimate includes several uncertainties and limitations in the exposure and hazard assessment. The main uncertainty is related to the hazard assessment; the HBGVs used were published before 2012 and thus do not consider toxicological data published later. Similarly, the information on toxicokinetics, including excreted urinary fractions (F_{UE}), was limited. For TCIPP and TCEP, F_{UE} was derived from TDCIPP data. In addition, the limit of detection (LOD) and limit of quantification (LOQ) values for the analytical methods were quite high in both biomonitoring and the food analyses. As regards the external dietary intake estimates, the occurrence data from Belgium might not reflect contamination on the European level.

3.10. Lead

The EBoD calculations took into account developmental neurotoxicity among children and premature mortality among adults (Mahiout et al., 2022b). For these endpoints, epidemiological data are available on the direct association between blood lead levels (BLL) and health effects in humans (Lanphear et al., 2005, 2018). The existing HBM exposure data gathered for the HBM4EU consisted of different Slovenian data (2008–2020) on children and adults, covering different regions including the hotspot in the Meža valley, Spanish data on adults (BIOAMBIENTES study, 2009), Czech data on children and adults (Czech HBM, 2015–2016), German data on children (GerES V, 2014–2017), and Belgian data on adults and adolescents (FLEHS I adults 2004–2005; FLEHS IV adolescents 2016–2020). The proportions of children with a BLL of >20 μg/L ranged from 0 to 89%. The GM for all BLL ranges varied

between the studies from 8 to 42 μg/L. The highest BLL was observed in the studied hotspot regions. Among adults, the proportions of those with a BLL of >10 μg/L ranged from 86 to 100%, with GM values from 23 to 37 μg/L.

Among the children and adolescents with a BLL of >20 μg/L, disability-adjusted life years (DALYs) ranged from 0 to 3437 per 100,000. In the BLL for children, the DALYs ranged from 1039 to 6326 per 100,000. Among adults with a BLL of >10 μg/L, the DALYs ranged from 310 to 733 per 100,000. The main uncertainties in the RA were related to either the representativeness of biomonitoring data or the dose–responses of the health effects. Since the exposure data were based on studies collected at different time periods (2004–2020) and the participants' age groups varied, the representativeness of this data to the current situation is uncertain. Although there is robust evidence on the association between developmental neurotoxicity and BLL, the dose–responses at low BLL levels (<20 μg/L) performed as sensitivity analyses include many uncertainties (Health Canada, 2013). Moreover, dose–responses related to overall mortality need to be confirmed by further studies.

3.11. Mercury

An RA of methylmercury (MeHg) exposure was conducted using published HBM data from European surveys with sampling between 1990 and 2017 for two populations groups, children/adolescents (aged 3 to 17) and women of childbearing age (aged 18 to 50) (Dominguez-Morueco et al., 2022). The RA strategy included estimations of the hazard quotient (HQ), based on the HBM-I value established by the German Human Biomonitoring Commission (Schulz et al., 2007) and the EU general population exceeding the tolerable weekly intake (TWI) (or their equivalent to TDI) defined by the European Food Safety Authority (EFSA) in 2012 (EFSA, 2012). In the first case, hair-Hg levels were converted into blood-Hg and in the second, TWI/TDI was converted into Hg hair levels, using earlier established correlations (Esteban-Lopez et al., 2022; FAO/WHO, 2004; WHO, 1990, 2008). The HQ approach showed that for both children/adolescents and women of childbearing age, the risk varied across EU countries and that some EU areas were close to or exceeded the exposure guidance value (Spain and Portugal, probably due to their higher seafood consumption). The results of the EFSA approach showed that the hair values of children/adolescents and women of childbearing age were below 1.9 μg/g, which is the hair level corresponding to a TWI of 1.3 μg/kg bw for MeHg (equivalent to a TDI of 0.19 μg/kg bw/d). Therefore in general, the European population does not exceed the daily average intake dose for MeHg/Hg. We identified high variability in the HBM data available on mercury, both in terms of the biomarkers/matrices used and the descriptive statistics reported. In addition, data from potentially relevant EU countries were missing. Therefore, RA could be further refined by accounting for differences in European exposure with harmonised and more widespread HBM data.

3.12. Mycotoxins

HBM data on deoxynivalenol (DON) was obtained by a bibliographical search (children, adolescents, and adults) and from the HBM4EU-aligned studies (adults) that provided data for France, Germany, Iceland, Luxembourg, Poland, and Portugal. The RA was conducted using two different approaches: comparing the HBM data with the TDI-based HBM-GV for DON (Mahiout et al., 2022b), and reverse dosimetry to back-calculate external exposure from the measured urinary DON levels and comparing it to the TDI (Alvito et al., 2022). As both these approaches used the urinary mass-balance method for conversion between external and internal levels, the results were very similar.

The HBM data obtained from the literature review showed that exposure to DON was generalised and affected different ages and specific groups of the population. The results obtained from the aligned studies conducted in France, Luxembourg, Poland, and Portugal showed that the highest percentiles of exposure (P90 and P95) represented a potential health concern, as the HQ obtained through comparison with the HBM-GV was above one (France: 1.24–1.64; Luxembourg: 1.09–1.42; Poland: 1.47–2.08; Portugal: 1.17–1.58, respectively for P90 and P95). However, the mean and median levels of exposure to total DON did not seem to represent a health concern (France: 11.8 and 6.5 μg/L; Luxembourg: 10.6 and 5.9 μg/L; Poland: 15.6 and 10.2 μg/L; Portugal: 11.6 and 8.0 μg/L, respectively for mean and median concentrations of total urinary DON). The results obtained for the remaining countries (Iceland and Germany) presented an HQ below one for all the percentiles of exposure, and thus did not represent a potential health concern.

The main uncertainty related to the use of HBM data in the RA of DON was associated with the fact that the first morning void samples used in the RA may not reflect total daily exposure, as DON levels can show significant within-day variation.

3.13. PAHs

Based on the HBM4EU-aligned studies, the geometric mean of the urinary 1-hydroxypyrene (1-OHPyr) among European adults ranged from 32.7 ng/g creatinine to 268.6 ng/g creatinine. This metabolite was considered a surrogate for PAH4: benzo(a)anthracene (BaA), benzo(b) fluoranthene (BbF), benzo(a)pyrene (BaP) and chrysene (CHR). The new data from the HBM4EU-aligned studies were in line with the urinary 1-OHPyr values previously reported in the literature (Sarigiannis and Karakitsios, 2019), with no significant difference; i.e., a pooled mean of 97 ± 44 ng/g creatinine compared to 113 ± 78 ng/g creatinine in the aligned studies ($p = 0.693$, Student's t-test) (Mahiout et al., 2022b).

1-hydroxypyrene levels were converted into pyrene (and PAH4) intake using PBPK modelling and ELCR was calculated on the basis of previously published dose–responses (ECHA, 2018). The ELCR calculated from the dietary exposure for the mean PAH4 intake was in the order of magnitude of 10^{-5} to 10^{-6}. The ELCR based on the PAH4 dietary intake data, estimated by EFSA (EFSA, 2008, 2015a), was one order of magnitude higher than those based on the PAH4 intakes that were reverse calculated from the HBM data.

These estimates have several uncertainties, the most significant being related to the applied dose–response relationship, based on studies of animal oral exposure to coal tar mixture. Whether 1-hydroxypyrene is a reliable bioindicator of measured dietary PAHs exposure under normal conditions (Viau et al., 2002) was also uncertain. The third issue was related to inhalation exposure, which needs to be properly addressed by, for example, using other urinary metabolites. Other metabolites should also be used to account for exposure to PAH mixtures.

3.14. Pesticides: chlorpyriphos

HBM4EU-aligned study data on urinary metabolite 3,5,6-trichloro-2-pyridinol (TCPy) levels were used in the chlorpyriphos RA (Tarazona et al., 2022b). U-TCPy was at detectable levels in most samples, with the exception of the French studies, which had detection frequencies below 5% for both children and adults. In the other studies, the 95th percentiles ranged within a factor of 6 (3.08–18.38 μg/l for children, 2.07–11.22 μg/l for adults). As an acceptable daily intake (ADI) cannot be established for chlorpyrifos due to genotoxicity concerns (EFSA, 2019), the RA was based on the Margin of Exposure (MoE) approach, comparing the Human Biomonitoring Points of Departure (HBM-PoD derived using the urinary mass-balance approach) with the 95th percentiles for each population, in order to cover the highly exposed population group. The results were grouped into four categories, defined on the basis of the standard assessment/uncertainty factors proposed by the EFSA and the ECHA.

We observed clear concerns regarding three child populations, with the MoEs below the standard assessment factors: 100 for PoDs based on

no effects, below 300 for the PoD based on observed effect (Lowest Observed Adverse Effect Levels – LOAEL), and below 10000 for the genotoxicity PoD. We also identified concerns regarding two adult populations as the MoEs were lower than those required for standard assessments with additional uncertainty factors (a factor of 10 for NOAEL to LOAEL, and a factor of 3 for subacute to subchronic extrapolation). Concerns for the other child and adult populations could not be ignored, as the MoEs were higher than those mentioned above but did not offer an additional margin of 10 (Tarazona et al., 2022b). The specific uncertainty related to the use of HBM data is that the metabolite used for biomonitoring also exists as a food residue, and its presence in urine may also be a consequence of the absorption and elimination of TCP present in food. This brings additional conservativeness to the assessment, as TCP has less toxicity than the active substances chlorpyriphos and chlorpyriphos methyl.

3.15. Pesticides: pyrethroid mixture

An overall RA of pyrethroids used HBM data and was based on measuring the biomarker 3-phenoxybenzoic acid (3-PBA), which is a common metabolite of many compounds from this group (Tarazona et al., 2022a). Under very conservative assumptions (i.e., the use of the ADI for lambda-cyhalothrin, which is the lowest among all the pyrethroids and the lowest known urinary excretion fraction of 9% for 3-PBA as observed with deltamethrin), screening values of 3.25 μg/L urine and 4.8 μ/L urine were established for children and adults (general population), respectively. The same screening values were used for the sum of 3-PBA and 4-FPBA which is a metabolite of fluorinated compounds such as cyfluthrin. For Tier I of the RA, urinary concentrations (P95 from aligned studies) were compared to these values.

Data on children's pyrethroid exposure were available in Belgium, Cyprus, France, Israel, the Netherlands, and Israel. Adults were sampled in France, Germany, Israel, and Switzerland. Whereas no risk was observed among adults, among children, P95 levels exceeded the screening value in all studies, with the highest value (sum of 3-PBA and 4-FPBA) obtained in Belgium (7.1 μg/L). This suggests pyrethroids are a potential health concern and that further refinement of the RA is warranted.

A probabilistic refinement was performed, taking account the differences in ADI (up to 20 times) between the various pyrethroid parent compounds and the variability in urinary excretion of 3-PBA with regard to individual substances. However, this approach still resulted in a potential risk among about 2% of Belgian children when using the worst-case assumption, that their exposure was due to the most hazardous pyrethroid with the lowest ADI (lambda-cyhalothrin). The risk of exceedance was reduced by 1–0.1% when assuming mixed exposure to several pyrethroids with different ADIs.

Tier II of the RA was based on the HBM-GVs derived for individual pyrethroids, in which more specific metabolites (biomarkers) were measured in urine (i.e., for bifenthrin, lambda-cyhalothrin, cypermethrin, deltamethrin, and permethrin). The 95th percentiles from the aligned studies were compared to these HBM-GVs but were consistently below, among both children and adults. This Tier II assessment confirmed that compounds with higher toxicity, i.e., lambda-cyhalothrin, cypermethrin, and tau-fluvalinate are not the main contributors to 3-PBA levels, and therefore they are not considered the main drivers of concerns raised in Tier I of the RA (Tarazona et al., 2022a).

There are several sources of uncertainty that must not be disregarded. (1) It is not always possible to derive an HBM-GV for every compound. (2) Even the more specific metabolites needed for Tier II of the RA may still be common to more than one substance, making it difficult to identify the individual source of exposure. (3) In kinetic studies of human volunteers, the estimates of the urinary excretion fraction of a metabolite are mostly based on a limited number of participants and always, of course, adults.

3.16. PFAS mixtures

The recent internal exposure data on European adolescents generated in the HBM4EU-aligned studies approach (dataset with N = 1957, sampling years 2014–2021) revealed that this population is exposed to Per- and polyfluoroalkyl substance (PFAS) mixtures. Mixture risk assessments of these PFAS mixture exposures were conducted for both median (P50) and highly exposed (P95) percentiles of the HBM4EU teenager study population, based on three hazard-based approaches: the HI approach, the sum value approach as used by the European Food Safety Authority (EFSA), and the Relative Potency Factor (RPF) approach (Bil et al., 2022, 2023).

The HI approach resulted in the highest risk estimates based on P95 exposure, up to an HI of 6.2 for immune effects, as seen in the French study population. This was followed by the RPF approach, for which the highest RCR was 4.3 in the Swedish study population based on P95 exposures. By using the sum value approach, the highest RCR based on P95 exposure was 1.8, observed in the Swedish study population. All three approaches indicated that the combined exposure to PFAS was too high and could result in a human health risk for a considerable fraction of individuals in the HBM4EU teenager study population, thereby confirming the recent EFSA scientific opinion. Long-term exceedance of the HBM-GV for PFASs is undesirable, and a justifiable reason to reduce human exposure to a level below this threshold.

The outcome of each risk assessment approach contained uncertainty related to assessment-specific built-in assumptions and limitations. These concerned the type of hazard data used (epidemiological data or animal toxicity data), possible differences in potency, and the number of PFASs that could be included in the mixture risk assessment. Furthermore, the HBM data that were obtained from studies relying on a relatively high limit of detection introduced considerable uncertainty to the risk estimates, and the remaining differences between the aligned studies, such as sampling period, sampling collection method, sampling matrix (blood serum vs blood plasma), age distribution, and other study characteristics, hampered the effective combination of databases without introducing this heterogeneity.

3.17. Phthalates mixture

The internal exposure data on European children and adolescents generated in the HBM4EU-aligned studies approach revealed that almost all children and adolescents are exposed to phthalate mixtures (Vogel et al., in press). As phthalates act in a dose-additive manner, a mixture risk assessment was performed (Lange et al., 2022). The risk of combined exposure to five anti-androgenic phthalates (i.e., BBzP, DEHP, DiBP, DnBP and DiNP) was determined using the HI approach. For this, HBM-GV_{GenPop} were utilised (Lange et al., 2021). For DiNP, a provisional value (HBM-$GV_{GenPop\text{-}MRA}$) was derived based on foetal testis testosterone suppression and multinucleated gonocytes, using the urinary mass-balance approach to convert external exposure into internal urinary metabolite levels (Apel et al., 2020; Lange et al., 2022). Precautionary factors of 5 and 10 were applied to account for other anti-androgenic substances not assessed, yielding adapted HI values of 0.1 and 0.2, respectively. Individual HIs were compared to risk thresholds of HI of >1, 0.2, 0.1. The MRA revealed that approximately 17% of European children and adolescents are at risk of combined exposures to the above five phthalates (HI > 1). Importantly, of those exceeding HI = 1, the majority (63%) would have gone unnoticed in the single substance risk assessments, as the exposure levels of the single substances were below the respective HBM-GVs (Lange et al., 2022). There are some uncertainties in the risk assessment related to the differences in the available HBM studies, i.e., different sampling periods, sample collection method, data quality of analytical results, age distribution, and other study characteristics. Other uncertainties lie in the toxicological endpoints upon which the HBM-GVs used for combined risk assessment are based.

3.18. UV filter benzophenone-3

The RA of benzophenone-3 was first based on the HBM data obtained from three published studies conducted in 2010–2013 (Rousselle et al., 2022) and then extended using data from the HBM4EU-aligned studies (sampling period 2014–2018) (Mahiout et al., 2022b). Urinary levels of BP-3 (P50, P75, P95, max levels) were compared to the provisional HBM-GV derived using the urinary mass-balance approach. Six of the aligned studies conducted from 2014 to 2018 in the HBM4EU used new measurements of benzophenones, including BP-3. Comparison of the RA of more recent data showed that the P50 and P95 were lower than the previous studies. In the six new studies, the highest RCR at P95 was 0.2, whereas in the previous assessment it had been 1.15. However, the highest exposed cohort in the previous assessment was not included in the aligned studies, whereas the published exposure levels in the other cohorts were similar to those in the aligned studies. In general, women had higher exposure levels than men in the new studies, but age or sample regime (spot, 24hr, or first morning samples) had no clear influence.

The assessment revealed notable differences in exposure between the groups, with the most highly exposed groups in the HBM4EU-aligned studies approaching (but not surpassing) the provisional HBM-GV for BP-3. It should be noted that as BP-3 has a relatively short half-life, so we cannot rule out that exposure levels would be even higher shortly after sunscreen use. These results were compared with the outcome of the most recent opinion of the Scientific Committee on Consumer Safety (SCCS) on BP-3, which used the exposure assessment based on estimated consumption and the same PoD as that used for the derivation of the provisional HBM-GV for the no-observed-adverse-effect level (NOAEL) setting (SCCS, 2021). This opinion proposed a decrease from 6% to 2.2% in the maximum permitted concentration of BP-3 in sunscreens due to the possible risk among highly exposed populations (Rousselle et al., 2022).

4. Discussion

As part of the HBM4EU, we performed risk assessments of the HBM4EU priority compounds, the primary aim of which was to exemplify how HBM data can be used in RA, to describe the challenges and how they can be overcome, and to identify whether RA based on biomonitoring data raises potential concerns about health risks of specific substances. Using these examples, we discuss the different opportunities, benefits, and pitfalls of using HBM in chemical risk assessments, to guide regulatory risk assessors, and to facilitate its use.

4.1. Benefits and challenges of using HBM data in risk assessment

4.1.1. Benefits

The main benefits of HBM are that it can inform us of the aggregated exposure from multiple sources and via multiple exposure routes. It can also inform us of individual variability due to genetic or behavioural reasons. HBM is especially useful in the case of highly bioaccumulating substances such as lead, cadmium or PFASs. Our work on PFASs explored different approaches to using HBM data in the risk assessment of these long half-life compounds and succeeded in reducing the uncertainty related to the build-up of PFASs in the body over a prolonged period. RA using HBM data was in line with the earlier EFSA risk assessment, which was based on external intake estimates (EFSA, 2020).

Benzophenones, used extensively as UV filters in sunscreens, are an example of a substance group for which dermal exposure is the most relevant exposure route. The BP-3 risk assessments in the Cosmetics Legislative Framework have always been based on calculated exposure, which uses the concentrations in cosmetic products, estimations of use, and dermal absorption to assess the Systemic Exposure Dose. Real data on systemic levels obtained using HBM, however, can greatly improve the reliability of these exposure estimates and show potential differences between the different populations caused by differences in consumption patterns. Our BP-3 RA showed that the exposure levels range from safe for most people up to close to an RCR of 1 for the highly exposed population and increased our confidence in the exposure assessment of SCCS, although some uncertainty still remained concerning the representativeness of the sampled population.

Hand contamination and dermal or hand-to-mouth exposure is also important in occupational contexts. In the case of diisocyanates, dermal exposure may contribute to respiratory sensitisation to diisocyanates, emphasising the importance of the use of HBM in the RA of diisocyanates (Mahiout et al., 2022b). Hand-to-mouth contamination is likely to contribute to the total U–Cr levels in the metal industry, and this needs to be considered when assessing the health risks of Cr(VI) exposure (Santonen et al., 2022; Viegas et al., 2022). In our RA of Cr(VI)-related lung cancer, this was considered a potential source of uncertainty (Mahiout et al., 2022a).

4.1.2. Challenges

The fact that HBM combines exposure from multiple sources can be both a benefit and a challenge. Phthalates are a good example of a group of substances with multiple sources of exposure of which the respective contribution to overall exposure can only be elucidated by specific study concepts and questionnaires. The HBM4EU's RA of combined exposure to five phthalates indicated a risk for children and adolescents (Lange et al., 2022). The HBM data were highly beneficial in revealing current internal exposure from various sources of several phthalates and in assessing the potential risk of phthalate mixture exposure. However, identification of the exposure sources and the possible contribution of unregulated sources requires further analyses of the exposure determinants (Martinsone et al., in preparation). In the HBM4EU, analyses assessed the exposure determinants of phthalates among children and adolescents in the HBM4EU-aligned studies, as well as in existing data sets on the adult population. Furthermore, an analysis of the time trends of phthalate exposure in Danish and German young adults offers insights into the effectiveness of recent phthalate regulations (Vogel et al., 2023).

Similarly, it is sometimes challenging to identify the contribution of occupational exposure when exposure from non-occupational sources is extensive. BPA is a good example of a case in which high background levels of total BPA in urine from food sources made the identification of occupational dermal exposure from cashier receipts difficult (Meslin et al., 2022; Ougier et al., 2021c). Although this worker group did not have higher exposure levels than the general population, some uncertainties remained about the potentially higher free BPA exposure via the skin than via oral exposure, which cannot be determined by measuring total urinary BPA levels (Meslin et al., 2022).

One of the main challenge in using HBM data in RAs is related to the availability of well-established, validated HBM methods. In their development, both adequate analytical aspects and information on toxicokinetics must be considered. Consideration should also be given to the specificity and sensitivity of the biomarkers in question. Partly because of these challenges, HBM methods are only available for a significantly lower number of substances than validated methods for environmental monitoring. Although it might be analytically possible to measure different contaminants in many different matrixes (in addition to commonly used blood and urine, e.g., hair, exhaled breath condensate, nails, saliva, breast milk, placenta, or semen have also been used), the available toxicokinetic data to link the measured levels to health effects or to external exposure may be too limited to enable proper interpretation of the results. For example, our RA of Cr(VI) (Mahiout et al., 2022a) was based on total urinary chromium, which is an unspecific biomarker reflecting exposure to both Cr(III) and Cr(VI). Although more specific biomarkers are available for Cr(VI), such as red blood cell (RBC)-Cr or exhaled breath condensate (EBC)-Cr(VI), measured or modelled (PBPK) data on the correlations between the external exposure and RBC-Cr or EBC-Cr(VI) levels is currently limited,

hampering the use of these data in RA (Leese et al., 2023; Ndaw et al., 2022; Santonen et al., 2022). Limited toxicokinetic data also caused challenges in, for example, the RA of pyrethroids, as information on the urinary excretion of the metabolites of some pyrethroids were based on data from only a single or very few individuals (Tarazona et al., 2022a). In the case of the pyrethroid bifenthrin, the F_{UE} was extrapolated from the data on lambda-cyhalothrin, which has a similar chemical structure, and gives the same metabolite ClF3CA (Tarazona et al., 2022a). Methylmercury is an important example of good epidemiological data linking hair-Hg levels to human adverse health effects. We used these data in our RA of methylmercury, which was based on hair-Hg measurements and blood-Hg levels (Dominguez-Morueco et al., 2022).

Sometimes, it might not be possible to measure the best biomarker from the RA perspective. A good example is RA of BPA: measurement of total urinary BPA instead of toxicologically active free BPA in the blood caused uncertainties, especially in the worker exposure scenarios involving dermal exposure (Ougier et al., 2021c). Metabolites shared by several substances may also cause challenges in the interpretation of the HBM data, as exemplified in the RAs of arsenic and pyrethroids. In the case of arsenic, dimethylarsinic acid (DMA) derived directly from food sources may result in overestimation of iAs exposure if iAs exposure assessment is based on the sum of As(III), As(V), MMA and DMA in urine (Buekers et al., 2023; Mahiout et al., 2022b). Similarly, in the RA of acrylamide, some uncertainty was introduced by the fact that the urinary metabolite AAMA may be produced also endogenously. In this case, we assumed that this source did not, however, result in a significant overestimation of risk, as our RA (based on HBM data) was very much in line with an earlier RA that used external exposure data (EFSA, 2015b).

Our case studies did not include any essential elements, like zinc, but in general using biomonitoring in their exposure assessment may be complicated by homeostatic control mechanisms (Poddalgoda et al., 2019).

4.2. Sources and representativeness of human biomonitoring data

HBM data is available from different sources for use in chemical RA. These sources include research studies published in the scientific literature, summary reports from population surveys (e.g., reports from the German Environmental Survey[2] or the USA National Reports on Human Exposure to Environmental Chemicals[3]) or summary reports on occupational biomonitoring data from national laboratories performing HBM analyses or from companies' health or exposure surveillance programmes. The HBM4EU dashboard[4] is a platform that contains summary statistics on HBM4EU priority chemicals from existing European general population HBM data collections, obtained through the HBM4EU project.

These data collections typically include summary statistics of biomarker levels including mean, median, P90, and P95 levels, confidence intervals (CIs), or ranges. These types of aggregated data are generally sufficient for RA purposes. The use of individual data may become relevant in the case of mixture risk assessments of several similarly acting substances. Unless the exposure to the different substances is highly correlated, using aggregated data for mixture RA will result in an overestimation of the risk, as it assumes that the highly exposed group is the same for all substances. Using individual data enables a more accurate RA. This kind of raw data may be available on request from researchers. For the RAs summarised in this publication, individual data were used in the combined RA of PFASs and phthalates (Bil et al., 2023; Lange et al., 2022).

As Table 1 shows, we primarily used data from HBM4EU-aligned studies (Gilles et al., 2021) in our RAs for the HBM4EU. The major benefit of the HBM4EU-aligned studies' data was that they included biomarker data from several European countries, measured in laboratories whose quality was guaranteed by its successful participation in the HBM4EU interlaboratory comparison and external quality assurance (ICI/EQUAS) scheme (Esteban Lopez et al., 2021). If the sampling was performed in the HBM4EU, harmonised methodologies (and questionnaires for background information) were used. The HBM4EU-aligned studies also included an analysis of samples from existing national biomonitoring programs or biobanks, like the German Environmental Specimen Bank (ESB) (Kolossa-Gehring et al., 2012) and the French Esteban study (Fillol et al., 2021).

Aligned data on several countries were not, however, available in all cases. For example, the aprotic solvents (NMP, NEP, DMF) RA was based on only two German studies – the ESB program and the GerES. Some risk or EBoD assessments, such as o-toluidine, lead and methylmercury assessments, were based solely on published data (Table 1). In the RAs focusing on occupational exposure (Cr(VI), o-toluidine, diisocyanates), we used either published data or database data from a national biomonitoring laboratory (FIOH's HBM database, not publicly available). The main challenges related to the use of these occupational biomonitoring database data are related to the limited availability of contextual data on the tasks covered and possible bias towards companies with good occupational safety and health practices, as small companies with less knowledge on monitoring practices and more poorly functioning occupational health services may not be included in monitoring programmes. More detailed contextual data may be available from individual research studies, but these are often limited in size and representativeness.

HBM data's representativity of the target population is one of the main issues that need to be considered when using this data for RA. HBM data provide specific information on the population to be investigated. They might be site- (Europe or, e.g., a hotspot),condition- or population group-specific (e.g., fish eaters, occupationally exposed, specific age groups). Data from small children (infants and toddlers) is rarely available, limiting the use of the HBM approach for the RA of small children. Biomonitoring studies carried out in some specific hotspot areas might not be suitable for EU-level RA purposes. In the HBM4EU-aligned studies of the general population, the requirements to participate were no recruitment in known hotspots and equal representation of both sexes. It was also recommended that the studies included participants with different SES and residents both from rural and urban areas (Fiddicke et al., 2021). The RA of methylmercury found significant country differences in hair mercury levels, likely reflecting differences in fish consumption patterns. This emphasises the importance of representative data from different regions/countries in Europe for a European-wide overview of methylmercury exposure. On the other hand, for many other substances, such country differences may not be particularly relevant. In such cases, also data from large North American HBM programs, such as NHANES[5] in the US and CHMS[6] in Canada, may provide valuable information for the European risk assessors, provided that use of the chemicals of interest can be considered similar between these regions.

In the case of aprotic solvents, HBM data were only available from Germany. However, in this case, RCRs – even for the combined exposure of three aprotic solvents using maximum levels measured in Germany –

[2] https://www.umweltbundesamt.de/en/topics/health/assessing-environmentally-related-health-risks.

[3] https://www.cdc.gov/exposurereport/index.html.

[4] https://www.hbm4eu.eu/what-we-do/european-hbm-platform/eu-hbm-dashboard/.

[5] NHANES (National Health and Nutrition Examination Survey): CDC (Centre for Disease Control and Prevention). Biomonitoring data tables for Environmental chemicals: https://www.cdc.gov/exposurereport/data_tables.html.

[6] CHMS (Canadian Health Measure Survey): Health Canada. https://www.canada.ca/en/health-canada/services/environmental-workplace-health/environmental-contaminants/human-biomonitoring-environmental-chemicals/canadian-health-measures-survey.html.

showed a maximum HI of 0.6 for young adults. Although investigations covering other European countries would be useful to confirm low levels in elsewhere, the German data may be sufficient to conclude that concern regarding these solvents is low, especially if this can be supported by other data (e.g. information on consumption or external levels in food or the environment, supporting low variability among the levels of different populations). The same was true in the RA of o-toluidine among the general population (Huuskonen et al., 2022). Although the HBM data were limited and covered individuals from only three countries, the estimated cancer risk of the general population was very low, at 1×10^{-8}.

When monitoring substances with fluctuating exposure patterns and/or rapid metabolism and short elimination half-life, the timing of the measurements is very important if single spot urine samples is used. We observed great variability in exposure to benzophenone (BP-3) among individuals, with females showing higher levels than men. As no information on the actual sunscreen use of these participants was available, it remained uncertain whether the HBM studies truly captured the highest exposures (Mahiout et al., 2022b; Rousselle et al., 2022). Similarly, the levels of BPA or the mycotoxin DON showed significant within-day variation, which caused some uncertainty in RA when spot samples were used (Martins et al., 2019, 2021; Meslin et al., 2022). On the other hand, first morning void samples of phthalates and bisphenols have previously shown to correlate well with the daily composite, indicating their feasibility for estimating daily exposure doses of urinary contaminants (Mok et al., 2022). Sampling time in relation to the time of exposure is also important in the occupational biomonitoring of short half-life substances. In occupational exposure, the levels of short half-life substances typically peak post-shift or in some cases, the next morning. Therefore, in occupational studies, typical sampling time is post shift. If the HBM-GVs for these compounds are based on steady state levels, this may bring some uncertainty to the RA – usually making the RA more conservative – as discussed in the case of o-toluidine RA (Huuskonen et al., 2022).

One important aspect related to assessing the risks of current exposures is the time between the sampling and the use of the data. In the case of lead, this introduced uncertainty to the RA due to the known decreasing time trend in BLL in Europe. As lead was not included in the HBM4EU-aligned studies, its health impact assessment used earlier-published blood-Pb data, collected in different countries over different time periods in the last 20 years. On the other hand, the global consumption of lead is increasing today, because of an increasing demand for energy-efficient vehicles (Rudnai, 2019). The same might also apply to the RA of phthalates, which used data gathered between 2014 and 2021. We observed that participants of studies with earlier sampling periods (2014, 2015, 2016) had significantly higher HI levels than participants of studies that collected samples in later years. However, geographical differences and differences within single data sets seem to be more pronounced than this time effect.

In the case of occupational exposure, the type of industry and processes might generally be more important than, for example, the country of location, especially if we consider risks in Europe, with harmonised OSH legislation. However, differences between background (environmental) exposure in different locations may exist, as shown in the case of cadmium (Berglund et al., 2015). These may also impact the levels observed among workers. Similarly, regarding cumulative substances, workers' age and former career may have an impact on observed levels. Underlying health conditions or physiological status (e.g. pregnancy) may be a source of variability in small cohorts with only few workers. Operating conditions and risk management measures (RMMs) may differ widely among companies, and it is important to be able to link the exposure to the process, operating conditions, and RMMs currently in place. Moreover, personal working habits and the effectiveness and proper use of personal protective equipment is a typical source of variability observed in exposure. In these cases, the quality of the contextual information collected is a key aspect that might be more relevant than the number of workers enrolled in the biomonitoring campaign. On the other hand, if the number of measurements is high (as in the Cr(VI) case study) and a consistent pattern of exposure is seen, the data are valuable for drawing general conclusions regarding the risk of the worker group, even though the contextual data are limited. In our diisocyanate RA, we observed highly variable exposure levels, resulting in a highly skewed distribution of HBM data in the sectors of interest. For this reason, deterministic assessment based on single-point estimates of exposure was not considered adequate, and the exposure assessment was based on the overall distribution of the data. Supplementary Table 3 lists the aspects that must be considered when evaluating the representativeness and applicability of occupational biomonitoring data for RA.

Overall, although some uncertainties might be related to the representativeness of HBM data, in many cases of RA, HBM data are often not the only source of exposure data, and even a dataset with limited representativeness can provide valuable support for RAs performed on the basis of external exposure/intake data. In many cases, the RAs performed under the HBM4EU were able to confirm the results of the earlier RAs that used external exposure estimates, reducing the related uncertainty. This was the case with, for example, the RAs of acrylamide, arsenic, and BP-3, all of which raised concerns about the representativeness of or some confounders in the HBM data.

4.3. Assessment of dataset quality and comparability

The quality of the available data is another aspect the risk assessor needs to consider when using HBM data in regulatory RA. In most RAs for the HBM4EU, the HBM4EU-aligned study data were mainly analysed by laboratories that succeeded in the HBM4EU ICI/EQUAS quality process (Esteban Lopez et al., 2021) or used equivalent quality assurance schemes. With some biomarker data, it was not possible to obtain comparable quality with the HBM4EU QA/QC efforts. Although this may bring some uncertainty to the assessment, it was, however, not usually considered a major source of uncertainty in any of the RAs performed. However, some differences between the analytical performance of the laboratories also related to their ability to quantify biomarkers at low concentrations. This must be taken into account, as it may impact statistical parameters if not all metabolites are measured with the same sensitivity in different laboratories. In addition to the quality of the analytics, the quality of pre-analytical (specimen collection, transport and processing) and post-analytical aspects [e.g. (Bonini et al., 2002; Plebani, 2006)] must also be considered. LaKind et al. (2014) have proposed general criteria for biomonitoring study quality that could be considered when assessing the quality of HBM data. The LaKind criteria were applied in, for example, the systematic review of existing (published) biomonitoring data on occupational exposure to diisocyanates and bisphenols (Bousoumah et al., 2021; Scholten et al., 2020), although it was observed that the application of these criteria to occupational biomonitoring studies might be challenging in some cases, due to, for example, the typically much lower number of participants than in general population studies. In addition to the LaKind criteria, Gallo et al. (2011) provide guidance on study reporting for observational research in the STROBE-ME statement (The STrengthening Reporting of OBservational studies in Epidemiology – Molecular Epidemiology). A summary of the main issues to be considered in relation to the quality of pre-analytical and analytical phases is provided in the supplementary material (Sources of pre-analytical and analytical uncertainties).

One aspect not considered in the LaKind criteria is related to the different practices for normalising urinary HBM data. When chemical concentration is dependent on urine production levels, it is often reported as either relative to urinary creatinine concentration, to correct for variable dilutions in spot samples (creatinine adjustment/correction), or in some cases as normalised to a certain average urine specific gravity (relative density) or osmolarity. Comparison of the results of multiple studies may therefore require unit conversions, which can use either the actual creatinine excretion data reported for the study

population, or if these are not available, the default values for average creatinine excretion. This was done in, for example, the RA of hexavalent chromium (Mahiout et al., 2022a) and diisocyanates (Mahiout et al., 2022b). Supplementary Table 5 provides more information and presents formulas that can assist in converting data between different units.

4.4. Availability of biological guidance and limit values for RA

When HBM data are used in RA for exposure assessment, risk characterisation is the most straightforward when up-to-date health-based biological guidance or limit values are available, and the user agrees on the hazard assessment made for setting the values. Health-based HBM-GVs have been derived for several substances within HBM4EU (Apel et al., 2020, 2022). Several biological guidance or limit values have also been derived by different national or international bodies. Supplementary Table 2 presents examples of these. Like health-based guidance or limit values for external intake, these values are regularly revised and often lowered on the basis of new scientific knowledge. A tool has been developed to help researchers, public health professionals, risk assessors, and regulatory decision-makers quickly locate relevant data on environmental chemicals. It consists of an online repository for international health-based guidance values to facilitate the interpretation of HBM data, referred to as the 'Human Biomonitoring Health-based Guidance Value Dashboard' (HB2GV) Dashboard[7]) (Nakayama et al., 2023). So far, more than 500 biomonitoring guidance values from 47 sources have been identified for this dashboard.

In our RAs for the HBM4EU, we preferred to use HBM-GVs derived within the HBM4EU, if such were available (Table 1). The RA of arsenic used the BE level set by Hays et al. (2010a) for non-cancer health endpoints, and the RA of methylmercury used the German HBM-I value (Dominguez-Morueco et al., 2022). In other cases, provisional HBM-GVs were derived for RA, or reverse calculation methods were used to convert internal levels into external exposure. The approaches available for converting internal levels into external levels (or vice versa) and for setting HBM-GVs have been summarised (Apel et al., 2020, 2022; Hays and Aylward, 2009; Hays et al., 2008). OECD guidance on the setting of occupational biomonitoring guidance values is also available (OECD, 2022). In our RAs, we typically used the urinary mass-balance approach (Apel et al., 2020; Hays and Aylward, 2009; Hays et al., 2008) to calculate provisional HBM-GVs from health-based guidance or limit values given for external intake (Table 1). The advantage of this approach is that it can be employed relatively easily with only minimal toxicokinetic information and can be used for screening purposes, for example, to obtain an overall view on the existence of health risks. It is also useful when supporting data are needed to complement external measurement and/or modelled data.

PBPK modelling for reverse or forward dosimetry was used for the RAs of PAHs, bisphenols, diisocyanates and o-toluidine. In the case of PAHs, the probable daily intake (PDI) of pyrene was estimated using the reverse-dosimetry of urinary 1-OHPyr. For the RA of diisocyanates, a specific PBPK model was created for MDI and 2,4/2,6-TDI, based on existing industrial hygienic and biomonitoring data (Scholten et al., submitted). This PBPK model was used to convert urinary MDA and TDA (metabolites of MDI and 2,4/2,6-TDI) levels into external exposure levels for assessing the risk of asthma related to the exposure to MDI and 2,4/2,6-TDI (Huuskonen et al., 2023). In the case of BPA and BPS, the RA was based on the HBM-GVs derived using existing PBPK models for these compounds (Meslin et al., 2022). In the RA of o-toluidine, both the urinary mass-balance approach and a general PBPK model in the INTEGRA platform was used to convert urinary total o-toluidine levels into external exposure levels for the assessment of cancer risk (Huuskonen et al., 2022). Both approaches resulted in estimates of external exposure that were close to each other.

Measured data on the correlations between external and internal levels are typically used for setting biological guidance or limit values corresponding to external (air) limit values in occupational settings. However, similar correlation data may be available also for setting of general population internal guidance or limit values (Health Canada, 2017; Poddalgoda et al., 2019). Such data can be obtained from either controlled exposure studies in volunteers or from studies measuring both external and internal levels in real life exposure settings. Measured correlation data from occupational settings were used in the RA of Cr (VI) (Mahiout et al., 2022a). For this RA, the total U–Cr levels were converted into inhalation exposure using the regression equations developed as part of the HBM4EU chromate study for welders and workers using soluble chromates in Cr(VI) surface treatment activities (Viegas et al., 2022). This enabled the assessment of workers' cancer risk.

In the case of some well-known cumulative substances, the RA or EBoD estimations can be directly based on reliable direct relationships between biomarker levels and adverse health effects, if these already exist. This was the case for lead, Hg, and cadmium. However, in these cases a link might need to be made between, for example, external exposure from different sources and internal effects, which would require substance-specific PBPK models. In the case of cadmium RA, PBPK modelling was necessary to account for its cumulative character by age to derive age-specific HBM-GV (Lamkarkach et al., 2021; Leconte et al., 2021).

4.5. Recognising uncertainties related to risk assessments

When performing RA, it is important to recognise the main uncertainties related to the data and their potential impact on risk characterisation. Uncertainties in the RA can be evaluated using different approaches, including both qualitative and quantitative approaches (see, e.g., (IPCS, 2008; WHO, IPCS, 2018). Uncertainties related specifically to the derivation of HBM-GV have been discussed by (Apel et al., 2020) who also describe an approach for assessing confidence related to the HBM-GV. A slightly modified approach is also presented in the OECD guidance (OECD, 2022), focusing on the setting of occupational biological limit values. In the RAs performed under HBM4EU, we have described uncertainties in a qualitative manner, identifying the potential significance and direction of the uncertainty. Table 2 lists the main uncertainties identified in the RAs. Even though some uncertainties are specifically related to the use of biomonitoring data and reverse/forward calculation of internal exposure and external exposure estimates, in many cases the greatest uncertainties were related to the dose–response of toxicological effects. Uncertainties related to the use of especially urinary HBM data have been discussed also earlier, for example use of spot urinary samples for short-lived substances, normalization of urinary data and inter- and intraindividual variation in excretion kinetics (Aylward et al., 2012, 2014; LaKind and Naiman, 2015). However, whether these potential sources of variability become relevant in RA, depends on, for example, how close the estimated exposure is compared to the health-based guidance or limit values, and on the availability of other supporting exposure data.

4.6. Use of exposure biomonitoring data to assess combined effects in specific chemical groups

We also applied HBM data in the assessment of combined exposure to specific mixtures. These included pyrethroids, PFASs and phthalates. In addition, we assessed the risk of PAHs, which are complex mixtures of hundreds of compounds.

The RA of phthalates employed the HI approach to assess the combined risk of five phthalates with a similar anti-androgenic mode of action. The RA used individual data on biomarker levels to avoid the possible over-conservativeness associated with the use of aggregated

[7] https://www.intlexposurescience.org/i-hbm/.

Table 2
Summary of main uncertainties identified in HBM4EU risk assessments.

Uncertainty	Comments and examples
Representativeness of data	Many substances had uncertainties related to the representativeness of the data. For example, in the case of lead, more recent HBM data may have resulted in a different EBoD estimation. In the case of BP-3, there were concerns related to the inclusion of the highest consumers of sunscreens. The RA of aprotic solvents was based on data from only Germany. The RA of diisocyanates was mainly based on occupational HBM data from Finland and may include some selection bias.
Uncertainties related to toxicological data or dose–responses	Many substances, such as organophosphate flame retardants, arsenic, diisocyanates and BPA, had significant uncertainties related to the toxicological data and dose–responses.
Uncertainty related to limited toxicokinetic data for reverse/ forward calculation[a]	Some pyrethroids, such as cyfluthrin or bifenthrin, had very limited or negligible information on the fraction of the substance excreted in the urine (F_{UE}). The same also applied to some organophosphate flame retardants, such as TCIPP and TCEP.
Shared metabolites or other sources of same metabolites[a]	In the case of arsenic, one analysed metabolite (DMA) may also be derived directly from food, and not only from the metabolism of inorganic arsenic. As regards pyrethroids, 3-PBA is a metabolite shared by several pyrethroids with variable toxicity. The acrylamide metabolite AAMA is also derived from endogenous production. Urinary chromium represents exposure to both the carcinogenic Cr(VI) and the far less hazardous Cr(III).
Limited sensitivity of method	In the case of BPA, it was not possible to separate occupational exposure to biologically active free BPA via the skin from urinary total BPA. In the case of diisocyanates, lower but still relevant risks were impossible to quantify due to high LOQs in many measurements.

[a] These are uncertainties specific to the HBM-based approach.

data (Lange et al., 2022). Pyrethroids are an interesting example, as many of their members share the common metabolite 3-PBA (3-phenoxybenzoic acid), which is a much used biomarker of pyrethroid exposure. Even the more specific pyrethroid biomarkers cover more than one substance. However, although pyrethroids share common neurotoxicological properties, their potency differs. Therefore, a tiered approach in the RA of pyrethroids was developed. The first tier used a 3-PBA metabolite for which a conservative 'screening level' HBM-GV was developed on the basis of the most potent pyrethroid (with the lowest ADI) and conservative estimate of F_{UE}. This first tier assessment was then further refined using probabilistic approaches and biomonitoring data on more specific metabolites, as described in (Tarazona et al., 2022a); enabling a combined RA of all relevant pyrethroids.

The RA of PFAS considered different PFAS potencies by applying an RPF approach as an alternative to HI or for the sum value approach proposed by EFSA for four PFASs (Bil et al., 2023). The RPF approach for PFASs assumes dose addition and sets the potency of the index compound PFOA for liver toxicity at 1. It also expresses the toxicity of the other compounds relative to this as relative potency factors (Bil et al., 2022, 2023). The results of the RAs and the advantages and disadvantages of the different approaches for mixture PFAS assessment are further discussed in the paper by (Bil et al., 2023).

In the case of PAHs, pyrene was used as an indicator substance for mixtures of PAHs. Pyrene Probable Daily Intake (PDI), estimated from urinary 1-hydroxypyrene levels, was used to estimate PAH4 intake, based on the relative proportion of pyrene and PAH4 derived from the EFSA data of food residue and food consumption country-specific data (EFSA, 2015a). ELCR was calculated on the basis of the ECHA-RAC dose–response relationship for PAH4 (ECHA, 2018).

These examples clearly demonstrate different options for conducting RAs considering the combined effects of chemically structurally and toxicologically related substances.

4.7. Use of effect biomarkers in risk assessment

Effect biomarkers may provide a tool for improving RA and may be especially useful in the case of RAs of mixtures. Effect biomarkers consist of the measurable biochemical or physiological effects or other alterations within an organism that can be associated with an established or possible health impairment or disease, thus linking the exposure to health effects (Zare Jeddi et al., 2021). Once human internal exposure to a chemical is shown, the complementary use of effect biomarkers can help bridge health consequences by providing data on pre-clinical manifestations of disease that can, in some cases, be prevented. The advantages and limitations of their application for RA purposes are listed in Supplementary Table 6.

Beta-2-microglobulin (β2M), and retinol-binding protein (RBP), despite their non-specificity to Cd exposure, are widely used as biomarkers of the kidney effects of Cd exposure and formed the basis for the HBM-GV for cadmium used in the RA of cadmium (Lamkarkach et al., 2021). Biomarkers of genotoxicity in blood, such as micronucleus induction in peripheral blood lymphocytes, have been associated with an increased risk of cancer (Boffetta et al., 2007; Bonassi et al., 2007). The use of effect biomarkers alongside exposure biomarkers in the recent HBM4EU chromates study in an occupational setting contributed to bridging the gap from exposure to early biological effects (Santonen et al., 2022; Tavares et al., 2022). These data can be used to support the reduction of the occupational limit values for Cr(VI), although they were not useable for the quantitative cancer RA of Cr(VI) (Mahiout et al., 2022a). Moreover, even receptor-based *in vitro* assays can be used as effect biomarkers in epidemiological studies. Thus, nuclear receptor activities such as estrogenic activity in a human blood sample can be linked to various health endpoints and offer hints on the chemical mixture(s) or mode(s) of action that lead to the disease/dysfunction (reviewed in (Vinggaard et al., 2021)).

For the application of effect biomarkers, a careful selection should consider its relation to the chemical exposure and key cellular/molecular effects, mechanistic rationale, the feasibility of sampling, sensitivity, potentially confounding factors, and importantly, its relevance for human health. A recent review on effect biomarkers for hexavalent chromium and cadmium concluded that because many effect biomarkers are not specific to these heavy metals, a combination of several biomarkers should be used to establish the relationship between exposure and specific health outcomes (Ventura et al., 2021). Complementing exposure biomarkers with mechanistically-based effect biomarkers has been suggested as a strategy to validate a selection of human effect biomarkers using adverse outcome pathways, which has been reported for phthalates and reproductive effects (Baken et al., 2019). The systematic, standardised, large-scale implementation of effect biomarkers in future HBM studies could complement exposure data with mechanistically-based biomarkers of early adverse effects, as suggested for the case study of bisphenol-related effect biomarkers and their mechanistic pathways following the adverse outcome pathway (AOP) framework (Mustieles et al., 2020).

5. Conclusions

HBM is a powerful tool for identifying the proportion of people exposed to levels at which health effects can no longer be discounted. As demonstrated with HBM4EU priority substances, HBM can be

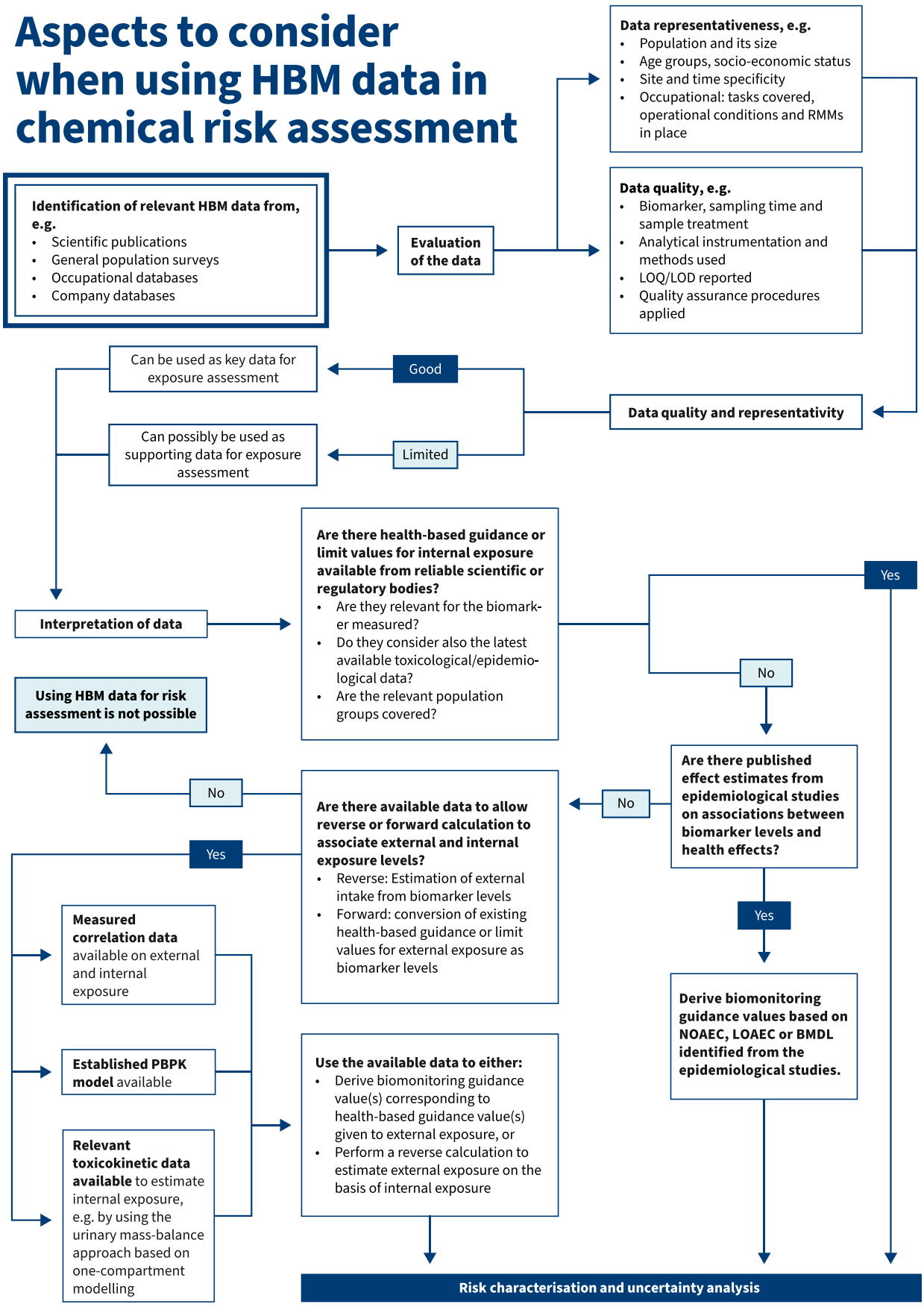

Fig. 1. Aspects to consider when using human biomonitoring data in chemical risk assessment. Detailed graphical presentations for the steps related to the derivation of biomonitoring guidance values can be found in, for example, Fig. 1 in (Apel et al., 2020) and Fig. 2 in (Gurusankar et al., 2017).

successfully used in RA, and its inclusion generally benefits RA, providing it with more confidence especially when used together with other exposure data. It is, however, important to consider the uncertainties involved. Although there are some specific uncertainties related to the use of HBM data, the main uncertainties are often not related to the use of the HBM data *per se*, but to the dose–response and toxicological data used, or the limitations in the size of the dataset, making them universal to RA in general. In fact, the inclusion of HBM data may often reduce uncertainty in RA if they are used to verify exposure assessments employing other methods, such as modelling. Fig. 1 summarises the aspects to consider when using HBM data in chemical risk assessment.

One of the major challenge in the use of HBM data in RA is the limited number of substances, for which biomonitoring methods are currently available. In addition, more specific and sensitive biomarkers are needed for more substances. The development of valid HBM methods and development of internal guidance or limit values to support the interpretation of HBM data requires good quality toxicokinetic data, which is currently not always available. In the future, HBM may have potential for wider use in RA when combined with *in vitro* and *in silico* methods to provide linkages between AOPs and human internal exposure levels. Naturally, this would also require continuing investment on biomonitoring programs able to provide representative HBM data covering relevant population groups.

As HBM provides information on populations' and individuals' total exposure to all sources via all exposure routes, it will be an important tool for the EU Chemicals Strategy for Sustainability, when moving towards the 'one substance, one assessment' policy in Europe. HBM can also offer insights into the time trends of exposure and vulnerable groups, and therefore provide information on the effectiveness of the implemented regulatory risk management measures.

Although the RA/EBoD work summarised here and in the specific publications is not intended to have direct regulatory implications, the results may be useful for policy-makers and for raising awareness of required policy actions, as newly generated HBM data, reflecting the current exposure of the EU population, has been used in many of the RA/EBoDs.

Declaration of competing interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2023.114139.

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