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ABSTRACT

Human health risk assessment is historically built upon animal testing, often following Organisation for Economic Co-operation and Development (OECD) test guidelines and exposure assessments. Using combinations of human relevant *in vitro* models, chemical analysis and computational (*in silico*) approaches bring advantages compared to animal studies. These include a greater focus on the human species and on molecular mechanisms and kinetics, identification of Adverse Outcome Pathways and downstream Key Events as well as the possibility of addressing susceptible populations and additional endpoints. Much of the advancement and progress made in the Next Generation Risk Assessment (NGRA) have been primarily focused on new approach methodologies (NAMs) and physiologically based kinetic (PBK) modelling without incorporating human biomonitoring (HBM). The integration of toxicokinetics (TK) and PBK modelling is an essential component of NGRA. PBK models are essential for describing in quantitative terms the TK processes with a focus on the effective dose at the expected target site. Furthermore, the need for PBK models is amplified by the increasing scientific and regulatory interest in aggregate and cumulative exposure as well as interactions of chemicals in mixtures. Since incorporating HBM data strengthens approaches and reduces uncertainties in risk assessment, here we elaborate on the integrated use of TK, PBK modelling and HBM in chemical risk assessment highlighting opportunities as well as challenges and limitations. Examples are provided where HBM and TK/PBK modelling can be used in both exposure

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assessment and hazard characterization shifting from external exposure and animal dose/response assays to animal-free, internal exposure-based NGRA.

1. Introduction

Risk assessment for human health due to exposure to chemicals is based on four pillars: hazard identification (the toxic effect), hazard characterisation (dose–response relationships), exposure assessment (quantification of the external and internal exposures), and risk characterization. In the risk characterization step, the collected data from the previous steps are integrated and analyzed to evaluate the potential health risk associated with exposure to chemical(s) (NRC, 2009). Quantifying the (potential) human health or environmental risks in a given exposure scenario is the most important step in the risk assessment process. Traditionally, hazard assessment (covering both hazard identification and characterization) has been based on animal studies with the derivation of a point of departure (POD). The POD can be derived either as the no-observed-adverse-effect level (NOAEL) or the statistical benchmark dose (BMD) and its lower confidence limit, the so-called BMDL. In the future, it is expected that there will be a reduction in the number of animal experiments in the risk assessment of chemicals (Balls, 2002; Birnbaum et al., 2016; Dura, 2021). In line with this trend, the European Commission adopted a new Chemicals Strategy in 2020, committing to the reduction of animal testing and promoting the replacement of such methods with alternative approaches (EC, 2020). Increased use of *in vitro* and *in silico* data for hazard identification and hazard characterization is expected to occur and the use of new approach methodologies (NAMs) will play an important role in future hazard assessment (Thomas et al., 2019; USEPA, 2021). NAMs is an umbrella term for many different methods (ECHA, 2016) e.g., *in silico*, *in chemico*, and *in vitro* methodologies (Wambaugh et al., 2019a). Several academic groups and large EU and US programs are working on the NAM concept (Åhs et al., 2022; EPAA, 2023; ICCVAM, 2023; Thomas et al., 2018; USEPA, 2021). Some NAMs are already available as Test Guidelines of the Organisation for Economic Cooperation and Development (OECD) and in use (ECHA, 2016; Kavlock et al., 2018; USEPA, 2018). The NAM approach not only aims to reduce, refine and (potentially) replace (the 3Rs) the use of animals for ethical reasons, but also follows economic (cost of animal studies) and scientific (human relevance) reasons, as well as public expectations. Additional outstanding scientific reasons are that traditional risk assessment methods cannot evaluate the safety of the increasing number of chemicals on the market in a timely manner (Herzler et al., 2021) and that they may have limitations in addressing issues such as susceptible populations (Hallier et al., 2002), aggregate exposure, and exposure to chemical mixtures (i.e. cumulative exposure) (de Jong et al., 2022; Luijten et al., 2023; Pallocca et al., 2022; Rotter et al., 2018).. Internal exposure estimation enables evaluation of simultaneous exposure to multiple chemicals and helps assessing the associated risk for human health (Bessems et al.). In fact, internal exposure reflects exposure from all sources and routes of exposure as a steady state concentration for chemicals with long half-lives or as concentration over-time for short-lived chemicals.

Shifting towards the use of internal concentrations to assess exposures to chemicals and their mixtures in human populations requires a comprehensive understanding of toxicokinetics (TK). TK investigates a compound's absorption, distribution, metabolism, and excretion from the body (ADME). TK processes are specific for each chemical and may vary depending on individual factors such as age, gender, ethnicity, and health conditions. TK data are vital for understanding (1) biotransformations of a chemical in the body; (2) the contribution of different routes of entry such as inhalation, dermal, and oral to the internal concentrations; and (3) the duration and intensity of internal exposure. Internal exposure can be estimated using an appropriate exposure biomarker (parent compound or phase I/II metabolite) in its corresponding matrices. This estimation is possible with different methods such as direct measurements, exposure-biomarker relationships, and kinetic models, depending on the available data and the specific requirements of the assessment.

The assessment of exposure by measuring internal concentrations of a chemical or its metabolites in the human body (e.g., blood, urine, hair) is defined as human biomonitoring (HBM) (Forschungsgemeinschaft and Angerer, 2002). HBM provides valuable information on the actual internal exposure and potential risks associated with exposure to specific chemicals. Internal concentrations can be used for exposure reconstruction using PBK models to infer the exposures that are likely to have resulted in the measured biomonitoring results (reverse dosimetry). Exposure reconstruction involves the integration of HBM data with other sources of information, such as TK data and exposure scenarios, and provides a better understanding of the relationship between external exposure and internal concentrations (Brown et al., 2015; Dopart and Friesen, 2017; Huuskonen et al., 2022). Thus, to complement HBM data, contextual information concerning exposure (e.g., exposure scenarios, risk management measures (RMM) in place) should be collected and described as detailed as possible.

The integration of NAMs into risk assessment, despite its challenges (ECHA, 2016; Westmoreland et al., 2022), is increasingly recognized in regulatory frameworks and supports a progressive shift towards Next Generation Risk Assessment (NGRA). For instance, the Roadmap for Action on NAMs in Risk Assessment of the European Food Safety Authority (EFSA) and the associated projects (e.g. ADME4NGRA) witness this conceptual evolution in the area of dietary exposure to chemicals, i.e., food safety within the EU (Cattaneo et al., 2023; Escher et al., 2022). A key aspect of this shift is the integration of physiologically based kinetic (PBK) modelling and HBM data in risk assessment (ZareJeddi et al., 2022). PBK mathematical models are a helpful tool for incorporating data from multiple routes and sources of exposure; these models are parameterized using *in vitro*, *in vivo*, and *in silico* methods facilitating the extrapolation of external exposure scenarios to generate internal exposure estimates at target organs over time. For instance, the use of PBK modelling in chemical risk assessment (CRA) has shown how these models can fill data gaps, reduce uncertainties, and enhance chemical safety evaluations (Apel et al., 2020).

An interdisciplinary network of experts in different regulatory and research roles under the umbrella of the European Regional Chapter of the International Society for Exposure Science (ISES Europe[1]) aims to advocate the use of biomonitoring in occupational and environmental health (ZareJeddi et al., 2022). In this paper, we stress the importance of integrating HBM data with TK and PBK modeling as standard practice in human health risk assessment. This study will evaluate three of the main factors necessary to achieve this goal:

- First, to show how HBM and toxicokinetics/PBK modelling are interlinked and can enhance each other.
- Second, to evaluate the potential of PBK modelling and HBM to serve as an effective approach in risk assessment.
- Third, to make recommendations for the implementation and integration of PBK modelling and HBM within existing frameworks for chemical risk assessment.

2. HBM and toxicokinetics

Several concepts have to be understood to use HBM to its full

[1] https://ises-europe.org/.

potential in exposure assessment (Viegas et al., 2020). Humans are exposed to chemicals from various routes (oral, dermal, and inhalation), and sources (consumer products, diet, and indoor/outdoor environment including workplaces). HBM provides an estimate of a population's exposure to chemicals from all possible sources and routes of exposure. These chemicals may have different effects on organs and systems in the body. Internal exposure at target organ or tissue is the gold standard of exposure assessment; however, this could be difficult to measure in human populations. Therefore, HBM using less invasive matrices such as blood and milk, and non-invasive matrices like urine and hair, is considered the best alternative and most appropriate tool for assessing human (co)exposure to chemicals,[23] (Manno et al., 2010; Zare Jeddi et al., 2022). Linking longitudinal HBM results with epidemiological and toxicological data can bridge exposures with health effects and inform on public health measures in place or to be adopted (Huuskonen et al., 2023; Santonen et al., 2023).

Human TK is particularly relevant in determining the sampling strategy in HBM campaigns (Claire and Sean, 2022; Coecke et al., 2013). TK data of a substance help in determining the most relevant specific biomarkers in HBM samples, i.e., parent compound or metabolites, and the most appropriate HBM matrix (e.g., whole blood, blood serum, urine) as well as the frequency and duration g of the sampling. Stanfield et al. (2022) employed a Bayesian methodology to infer ranges of exposure combining urinary measures of parent chemicals from the (US CDC) NHANES and other relevant sources with chemical metabolism information. Their study provided valuable insights into the mapping of parent chemical-metabolite relationships, contributing to the refinement of HBM strategies. The different routes of exposure have their own toxicokinetic and toxicodynamic characteristics, which may have impact on the appropriate sampling strategy. Information on absorption rates, metabolites formed, degree of excretion on a molar or mass basis, and half-lives helps to identify the most appropriate exposure window of particular biomarkers, i.e., the timeframe of exposure that is reflected in the measurement. Distinct chemical properties exert influence over human exposure and the toxicokinetics of chemicals with different properties. Li et al. (2019) explored the relative importance of near-field and far-field exposure routes for organic chemicals released to indoor air. Their external exposure model results indicated that dietary and nondietary ingestion dominate human exposure to hydrophobic chemicals of relatively low volatility, while inhalation of indoor air is the primary exposure route for volatile chemicals. Other routes, such as dermal and oral routes via drinking water, contribute relatively less to human exposure. Olsen et al. (2023) investigated the dosimetric relationship between exposure to a chemical contaminant and its concentrations in blood and urine. They used a toxicokinetic model to quantify the absorption and elimination of chemicals and found that the dose-to-concentration ratio depends on fundamental chemical properties such as partition coefficients and biotransformation half-lives. Chemicals with low volatility and moderate to high hydrophobicity exhibited higher concentrations in the blood, while chemicals undergoing significant biotransformation showed lower concentrations in blood compared to those with negligible biotransformation but similar partitioning properties. Chemicals with high hydrophilicity had the highest concentrations in urine. These property dependencies were observed across different age groups and body weights.

Choosing one or more specific biomarkers of exposure (i.e., the parent compound, the metabolites or both) that are robust and sensitive enough to quantify external exposures is essential as a poor choice in biomarkers could result in over or under estimation of exposures (Kolossa-Gehring et al., 2017). Note that some substances form the same metabolites, which will not provide a compound specific biomarker of exposure and the respective exposure levels. One such case is for di(2-ethylhexyl) adipate, whose major metabolite is adipic acid, a non-specific biomarker (Nehring et al., 2020). Relevant biomarkers can be identified reviewing chemical structures and postulating potential biomarkers from animal metabolism studies (Zbinden, 1991), and human *in vitro* and *in vivo* metabolism studies (Koch et al., 2013b; Nehring et al., 2019; Schütze et al., 2012; Wrobel et al., 2022). Human metabolism studies have been conducted, inter alia, for phthalates and their substitutes (Koch et al. 2014a; Lessmann et al., 2016; Schütze et al., 2017), solvents (Koch et al. 2014a), cosmetics additives/UV-filters (Bury et al. 2019a; Bury et al. 2019b), plant-protection products like pyrethroids (Schettgen et al., 2016), and also for pharmaceuticals such as paracetamol that can accumulate in humans from non-intentional exposure (David et al., 2021). For example, in a ten-year joint project between the German Ministry for the Environment and the German Chemical Industry Association, human metabolism studies conducted to investigate 50 new substances of interest, such as plasticizer substitutes, novel UV-filters, solvents, and other prioritized chemicals, spurred the development of new and specific analytical methods for HBM that could then be applied in suitable population surveys and in the derivation of toxicologically-based HBM values for seven chemicals (Kolossa-Gehring et al., 2017). At present, many *in silico* structure-based tools are available to predict the metabolism of chemicals (Litsa et al., 2021; Tyzack and Kirchmair, 2019). These tools help generating suspect screening lists to guide data analysis (Boyce et al., 2023) from *in vitro* and *in vivo* metabolism studies and are recommended to interpret HBM data (Daiber et al., 2024; Steckling et al., 2018).

As mentioned, understanding the TK of a compound is essential for developing the HBM sampling strategy campaign, including information on choosing the sampling times and the appropriate number of samples to collect (in particular for non-persistent chemicals with short half-life) e.g., for occupational studies and consumer products used at specific times of day or year (e.g., summer for using UV protection products) (Koch et al., 2014b). This can reduce the number of samples required to have a representative dataset to evaluate exposures, reduce costs and reduce the burden of the study for participants (Connolly et al., 2018; Kohsuwan et al., 2022; Scher et al., 2007). HBM data for a chemical are the result of aggregate exposure to that chemical from all possible source and routes of exposure. For example, an HBM intervention study on high molecular weight (HMW) phthalates (Koch et al., 2013a; Koch et al., 2013b) showed that the main exposure route for HMW phthalates predominately ingestion, while for low molecular weight (LMW) phthalates major routes of exposure (>50 %) seemed to be inhalation/dermal (Weschler et al., 2015). Thus, authors concluded that food contamination needed to be reduced to significantly reduce overall HMW phthalate exposure, while all sources (including indoor sources) were relevant for LMW phthalates. A similar approach has been successfully undertaken for BPA, identifying contaminated foodstuff as the major source of BPA exposure (Christensen et al., 2012). In a duplicate diet study that analyzed plasticizers in a total daily diet, and in parallel analyzed for urinary biomarkers of the participants, the comparison of the two intake estimates enabled the identification of the dominant intake source. For some plasticizers food ingestion was the dominant source (agreeing food intake estimates with HBM data), whereas for other phthalates other non-foodstuff sources contributed the dominant part of the total intake (Fromme et al., 2013). This type of information could support strategies and policies for RMMs, such as dedicated policies in different regulatory frameworks. In occupational settings, typical strategies are to collect pre- and post-work shift samples (Connolly et al., 2017), or for chemical substances that may accumulate over a workweek one urine sample at the beginning of the work week pre-shift and one urine sample at the end of the week post-shift. Next morning samples are used to identify delayed excretion kinetics (Jones et al., 2022). Also 24 h urine samples can be collected, although they are less commonly used (Connolly et al., 2018). These types of sampling strategies will provide an overview and potential accumulation of the exposure over the work period (Santonen

[2] https://www.cdc.gov/nchs/nhanes/index.htm.

[3] https://www.oecd.org/env/ehs/risk-assessment/occupational-biomonitoring.htm.

et al., 2022).

With the vast increase of initiatives supporting the development of HBM (e.g., HBM4EU,[4] PARC,[5] OECD[6]) it is essential to have appropriate and harmonized means for conducting and interpreting these datasets, and at the same time have fit for purpose approaches depending on the differences between compounds of interest and regulatory needs. Direct HBM data interpretation for risk assessment purposes is possible by comparing the concentration of the biomarkers detected in the different matrices to accepted levels of the specific indicators, i.e., the human biomonitoring guidance values (HBM-GVs), as estimated with information from forward dosimetry in TK, in compliance to the external acceptable levels of exposure (e.g., HBGVs). HBM-GVs correspond to the concentration of a chemical in a human biological matrix (e.g., urine, blood, hair) at and below which adverse health effects related to the chemical exposure are not to be expected to occur, according to the current knowledge. HBM-GVs for occupational exposures are called biological limit values (BLV).[7] HBM-GVs may considerably reduce the uncertainty in human health risk assessment classically performed solely on external exposure estimates (Lamkarkach et al., 2022; Lamkarkach et al., 2021). Also, they can easily be used to communicate potential public health risks to policymakers (Apel et al., 2020).

In 2017–2021, European Joint Programme HBM4EU produced a harmonized methodology to derive HBM-GV (Ganzleben et al., 2017) and derived a set of new HBM-GVs for the project's priority substances for the general population and workers (Apel et al., 2023; Apel et al., 2020; Lamkarkach et al., 2021; Lange et al., 2021; Meslin et al., 2022). This work is continued in the PARC project (Partnership for the Assessment of Risks from Chemicals) (Marx-Stoelting et al., 2023; PARC 2023a; b). The i-HBM Working Group of the International Society of Exposure Science (ISES) is periodically updating an inventory of HBM-GV derived by different organizations[8] (Nakayama et al., 2023). The list contains general population guidance values (GVs) for 140 chemicals of which 81 % are biomonitoring equivalents (BEs) and 14 % HBM-GV. BEs are derived from existing external exposure HBGVs for the general population, with no further appraisal of toxicity or epidemiological data (Angerer et al., 2011; Hays and Aylward, 2009; Hays et al., 2008; Hays et al., 2007) (Angerer et al., 2011; Bevan et al., 2012; Hays and Aylward, 2009). In the HBM4EU initiative, the preferred method to establish HBM-GVs is based on the relationship between biomarker levels and the most sensitive health effects (Apel et al., 2020). If human data are not available or sufficient to derive an HBM-GV, then an HBGV (e.g, TDI) can be used to derive a BE. It is worth noting that the HBM-GV calculation frequently assumes that the steady-state is reached for a substance in a certain biological matrix. In the absence of human internal exposure data, HBM-GVs can be derived from external exposure GVs or limit values using external-internal exposure associations or PBK modelling (Fig. 1: the link between external GVs and internal GVs, with the latter representing HBM-GVs). PBK modelling may reduce the use of uncertainty factors applied to the HBM-GV derivation which are not arbitrary but mathematically imprecise (Dankovic et al., 2015). In the absence of external GVs, HBM-GVs can be derived from a POD in an experimental (animal) study (Fig. 1: the link between POD and HBM-GV). In the occupational field, other derivation methods are explained in an OECD guidance document (GD) on occupational biomonitoring.[9] The very low number of HBM-GVs and BEs is one of the main obstacles of using HBM data in risk assessment of chemicals (Apel et al., 2020; Louro et al., 2019). Yet, the rapid progress in the HBM4EU project has demonstrated that the generation of a considerable number of new HBM-GVs can be achieved in a rather short time, if reliable hazard characterization data, and relevant TK data, PBK models and HBM data are present for the respective substances (Apel et al., 2023).

3. HBM and PBK modelling

PBK modelling is a necessary tool for the establishment of HBM-GVs for a specific chemical in compliance with the external HBGV or the POD. A PBK model is a set of mathematical equations that describe in quantitative terms the ADME processes of a chemical in the body. As a result, a PBK model predicts the time course of a chemical in an organism and the concentration at the target tissue or in a relevant biological matrix following human or animal exposure to that chemical. Fig. 1 shows where PBK modelling can be integrated in exposure assessment and hazard characterization. Fig. 1 also shows how HBM and HBM-GV are combined to calculate the risk characterization ratio (RCR) in risk characterization, similarly to RCR for external exposure. PBK modelling can be conducted using various commercial software such as Simcyp/SIVA,[10] Gastroplus,[11] or open-source software, PK-Sim,[12] IndusChemFate[13] and httk,[14] among others that are freely available. Input data for PBK models are physiological data of the exposed species (e.g., organ volumes, cardiac outputs to the different organs, urine excretion rate), as well as chemical-specific parameters, such as physicochemical (e.g., blood-tissue partition coefficients) and TK properties (e.g., absorption rate, fraction unbound in plasma and tissues, renal clearance, metabolic clearance). Parameters can be obtained from *in vivo, in vitro,* and *in silico* studies. A comprehensive list of available PBK software and tools to parameterize PBK models is available in the Supplemental Excel Sheet of Chang et al. (Chang et al., 2022). Results from a similar mapping exercise are also available from the International Society for Exposure Science European reginal chapter (ISES Europe) exposure modelling working group (Schlüter et al., 2022) and Madden et al. (Madden et al., 2020).

It is worth noting that the uncertainties in the *in vitro* and *in silico* methods used to generate these parameters and the inter- and intra-species variability of *in vivo* data lead to uncertainties in the PBK model predictions. Both variability and uncertainty analyses are important considerations in PBK modeling, especially if models are used in regulatory assessments, as they provide a measure of confidence in the model predictions (OECD, 2021). Variability is inherent to the natural difference or variations observed among individuals in terms of physiological factors (e.g. body weight, cardiac output) and cannot be eliminated. In PBK modeling, variability is incorporated by considering a distribution of parameter values rather than assuming a single fixed value for each parameter and using approaches such as Monte Carlo sampling (OECD, 2021). The distribution of physiological parameters can be set for specific ethnic populations and subpopulations (e.g. adults, children, males, females) (Ring et al., 2017). Such an approach leads to a more realistic distribution of internal concentration estimates and allows the identification of potentially sensitive subpopulations. Uncertainty refers to the lack of knowledge or the presence of potential errors in the input parameters (e.g. *in vitro* experimental and measurement errors, *in silico* calculation error), assumptions, or model structure used in PBK modeling (OECD, 2021). Like for variability, uncertainty analysis is carried out informing distributions around input parameters and generating probabilistic PBK models. Wambaugh et al., (2019b)

[4] https://www.hbm4eu.eu/.
[5] https://www.eu-parc.eu/.
[6] https://www.oecd.org/chemicalsafety/risk-assessment/occupational-biomonitoring-guidance-document.pdf.
[7] https://inis.iaea.org/search/search.aspx?orig_q=RN:52095954.
[8] https://biomonitoring.shinyapps.io/guidance/.
[9] https://www.oecd.org/env/ehs/risk-assessment/occupational-biomonitoring.htm.
[10] https://www.certara.com/software/simcyp-in-vitro-data-analysis-toolkit-siva/.
[11] https://www.simulations-plus.com/software/gastroplus/pbpk-software/.
[12] https://www.open-systems-pharmacology.org/.
[13] https://cefic-lri.org/toolbox/induschemfate/.
[14] https://cran.r-project.org/web/packages/httk/index.html.

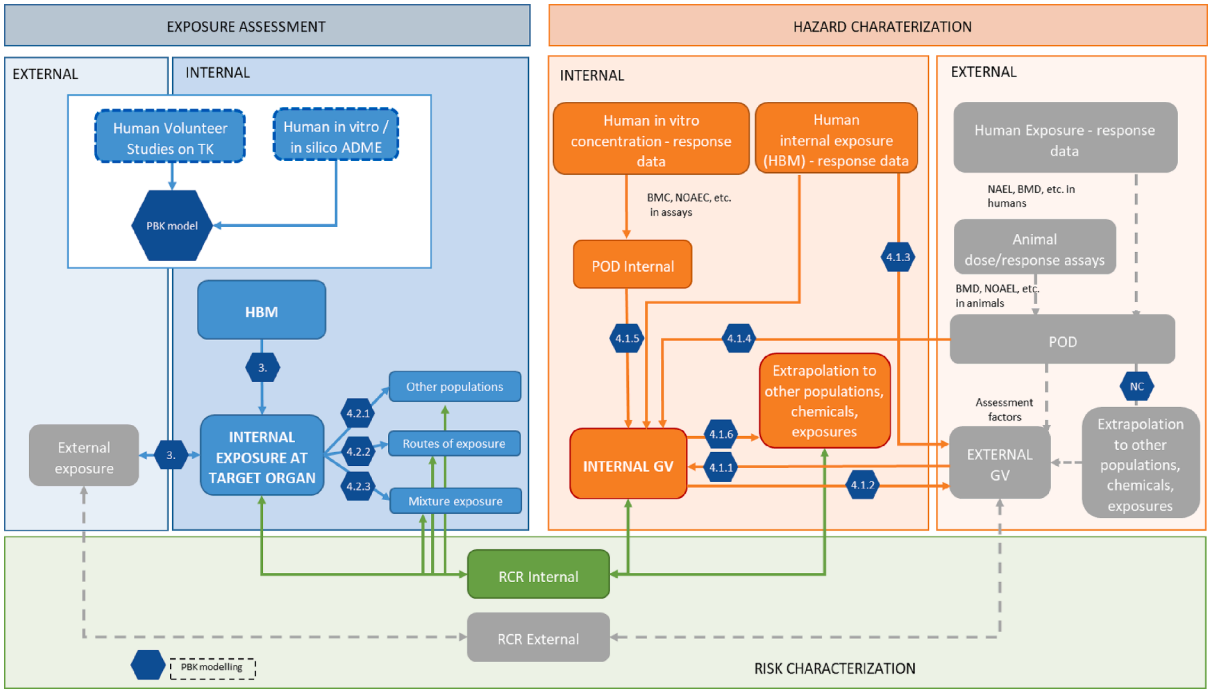

Fig. 1. Risk assessment shifting from external exposure and animal dose/response assays to next generation animal-free, internal exposure-based risk assessment. The arrows show the link between different steps in risk assessment. The blue hexagon shapes indicate PBK modelling. Some examples of possible applications of PBK modelling are given (numbers in the hexagons refer to the manuscript chapters; NC, PBK modelling application is not in the scope of NGRA). Data necessary to parameterize PBK models are shown in the white background rectangle. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

used Bayesian methods to determine uncertainty estimates for chemical-specific parameters (i.e. *in vitro* fraction unbound in plasma and intrinsic hepatic clearance (CLint), followed by Monte Carlo simulations to account for variability-only, uncertainty-only, and both uncertainty and variability. Carrying out separate variability and uncertainty analysis showed that for most of the studied chemicals the population variability contributes in larger extent than uncertainty to model output distributions. In general, parameters that exhibit significant uncertainty and most influence model output are the CLint and the fraction unbound in plasma (fu,p). CLint values can differ by more than one order magnitude due to the specific *in vitro* protocols used in different experiments (Louisse et al., 2020). It is worth noting that while variability cannot be eliminated, uncertainty can be reduced by improved data. A modified protocol for determining *in vitro* fu,p at different plasma protein concentrations significantly reduced the uncertainty associated with fu,p measurements (Wambaugh et al., 2019b). Overall, these findings emphasize the importance of setting harmonized *in vitro* methods to generate robust TK parameter values for PBK models.

Extrapolations are possible by modifying the model's parameters. For example, physiological and TK parameters can be adapted to extrapolate predictions for different species, as well as for different ages and health conditions, e.g., babies, older individuals (Hopf et al., 2012), and pregnant women (Louisse et al., 2010). Physico-chemical and TK parameters can be modified to extrapolate a chemical's PBK model to other chemicals with similar TK and mode of action (this being a read across approach) (Louisse et al., 2010). Also, extrapolation for different routes of exposure and different exposure scenarios is possible, as PBK models include equations that represent one or more routes of exposure. For example, a human oral PBK model can be adapted to represent dermal exposure, predicting blood concentrations upon dermal exposure to the chemical for which the oral model was initially developed (route-to-route extrapolation) (Bessems et al., 2017). A PBK model validated on human data based on a specific exposure scenario can be adapted to represent an occupational exposure scenario with different workload and exposure duration (scenario-to-scenario extrapolation), or the same scenario but at a different dose (dose-to-dose). Model predictions of internal exposures from external exposure data are called forward dosimetry.

PBK models can also be used for the reversed process, i.e., the reverse dosimetry, predicting external exposure concentrations from internal concentrations of biomarkers of exposure. For example, reverse dosimetry can estimate the external exposure that would result from a specific concentration at target tissue or a measured concentration of a biomarker of exposure in different matrices.[15] This approach is particularly important for *in vitro* – *in vivo* extrapolation (IVIVE): PBK modelling can extrapolate *in vitro* effect readouts (*in vitro* concentration–response results at tissue or cellular level) to the *in vivo* situation (*in vivo* dose–response data). Chang et al. (Chang et al., 2022) (see supplementary material therein) summarizes commonly used terms of IVIVE approaches that are relevant also to TK. The *in vivo* data estimated from *in vitro* concentration–response assays represent concentrations at target tissue. These target tissue concentrations can be correlated with internal concentrations through PBK modelling, which are commonly measured in blood and urine. With proper evaluation of the uncertainties derived from *in vitro* data and PBK model predictions, this approach allows the generation of HBM-GV. IVIVE procedures, when applied in PBK modeling, can address data gaps for "data-poor" chemicals (Breen et al., 2021). The use of generic PBK models, such as the httk[16] method, enables the extrapolation of *in vitro* toxicokinetic data for rapid chemical screening and prioritization. The extrapolation of metabolism data from *in vitro* experiments to *in vivo* clearances is crucial in toxicokinetics for assessing the risks associated with specific chemicals. Different toxicokinetic models are used for various extrapolation

[15] https://www.epa.gov/expobox/exposure-assessment-tools-approaches-exposure-reconstruction-biomonitoring-and-reverse.
[16] https://cran.r-project.org/web/packages/httk/index.html.

goals, such as hepatic blood clearance, organ clearance, whole-body clearance, and clearance at the level of hepatocytes. Krause and Goss (2018) developed a comprehensive toolbox for *in vitro-in vivo* extrapolation (IVIVE) of hepatic metabolism, including a sensitivity analysis to identify parameters that significantly impact the accuracy of the extrapolation results (i.e. *in vitro* clearance value, metabolically active components, unbound fractions, and partition coefficient). IVIVE relies on the assumption that *in vitro* metabolism data accurately represents *in vivo* clearance, assuming similar metabolic pathways and enzymes between *in vitro* and *in vivo* systems. However, uncertainties exist that can affect IVIVE predictions, including differences in measured intrinsic clearance between *in vitro* and *in vivo* systems and variations in the unbound fraction of chemicals due to binding to plasma proteins (Krause and Goss, 2021).

To compare IVIVE extrapolated data with direct *in vivo* measurements, researchers analyze key toxicokinetic parameters such as oral bioavailability, clearance, volume of distribution (Vd), and uncertainty. Wambaugh et al. (2018) conducted *in vivo* rat experiments for non-pharmaceutical chemicals and found that predictions for bioavailability were ineffective, while total clearance was generally underestimated. However, predictions for steady state, peak, and time-integrated plasma concentrations were reasonably accurate, and incorporating experimental measurements of bioavailability improved plasma concentration predictions.

High-quality HBM data are essential to validate human PBK model predictions but are affected by interindividual variability (Aylward et al., 2014). It is possible to add a distribution to each PBK parameter to account for this (McNally et al., 2021). Societal and regulatory requirements usually drive compound-specific models. Large targeted HBM campaigns can greatly facilitate model parameterization for compound-specific models. There is a positive feedback loop whereby PBK models (e.g., via sensitivity analysis) highlight specific areas where focused efforts should produce better TK data that, in turn, improve the model predictions.

Models are more or less complex depending on compound properties and parameter availability. More detailed predictions can be made for single chemicals when more data are incorporated into a model, whereas general predictions are made for large groups of data-poor chemicals (as in high-throughput bioactivity screening) (Chang et al., 2022). The higher-throughput approaches have the advantage for speed and flexibility but have limitations e.g., model evaluation. Pletz et al. (Pletz et al., 2020) investigated the suitability and limitations of generic PBK models (httk[17] and IndusChemFate[18]) in deriving HBM-GV values for several compounds with a view to facilitating the use of HBM data in the assessment of chemical mixtures at a screening level. The analysis showed that the application of PBK models provides a better understanding and interpretation of HBM data. However, it also showed limitations e.g., establishing safety threshold levels in urine is a difficult and complex task. The approach might be more straightforward for more persistent chemicals that are analyzed as parent compounds in blood (Pletz et al., 2020).

4. Integrating PBK modelling and HBM in risk assessment

The potential advantages of integrating TK data and modelling in human risk assessment based on HBM are numerous. For example, in hazard characterization PBK modelling can derive HBM-GVs from *in vitro* and *in vivo* dose–response studies, as well as from external HBGVs, replacing the use of some default extrapolation factors used in route-to-route or interspecies extrapolations. In exposure assessment, PBK modelling can predict internal concentrations based on external exposure (and vice-versa), improve the interpretation of HBM data, e.g., by accounting for internal concentration variability due to factors such as age and gender, and can extrapolate HBM data to other populations or different exposure scenarios.

4.1. Hazard characterization: Deriving HBM-GV and BE

4.1.1. Deriving BEs and BLVs from established HBGVs

Forward dosimetry using a PBK model for a chemical with an HBGV can predict the corresponding internal concentration of a specific metabolite or parent compound in a biological matrix (bioequivalent). This approach was used to derive an HBM-GV for bisphenol A (BPA) in the general population by Ougier et al. (2021). An existing PBK model for BPA was used to calculate BPA concentrations in urine considering steady state exposure at the tolerable daily intake (TDI) established by EFSA, and assuming constant exposure to BPA only through the oral route. In the case of o-toluidine risk assessment, BE were derived by using a urinary mass balance approach and a general PBK model (Huuskonen et al., 2022). O-toluidine is in the candidate list for authorization under REACH regulation,[19][20] and this example could facilitate the use of HBM data in possible future authorization processes.

In the occupational context, BLVs are generally derived by using measured correlation data to convert external levels to internal ones. However, often there is a poor correlation between external and internal occupational exposure levels, e.g., due to skin exposure contributing to the overall internal concentration in addition to inhalation exposure, or when the correlation was not based on personal air sampling, but rather on space sampling, or the use of respiratory protection, and the role of the ingestion route due to hand-to-mouth contact. PBK models can help to predict the probability of different routes of entry and derive the appropriate HBM value. The Scientific Committee on Occupational Exposure Limits (SCOEL) used PBK modelling to set BLVs for 2-methoxyethanol and its acetate, which are very well absorbed through the skin.[21] This approach was also used within HBM4EU project for cashiers' BPA exposure through the handling of thermal papers, resulting in BPA exposure via dermal absorption due to skin contamination (Ougier et al., 2021). However, the HBM-GV value for urinary BPA in occupationally exposed adults was lower than the 95th percentile of urinary total BPA distributions in the general population. Therefore, no HBM-GV was recommended for occupational health risk assessments and for identifying risky occupational exposures.

A PBK model for arsenic exposure has been reported (Mann et al., 1996) and was validated for inhalation exposure using data on urinary excretion after occupational exposure to arsenic trioxide dust and fumes. The model was adapted to humans from an animal model, with adjustments for body weight, metabolic rates, and absorption rates. The model describes ADME for the four major urine metabolites for inorganic arsenic (i.e., arsenate, arsenite, methyl arsonate, and dimethyl arsinate). The model was used to predict urinary inorganic arsenic metabolites from an inhalation exposure of 10 μg/m^3 (8 h time weighted average (TWA), the then current ACGIH Threshold Limit Value) inorganic arsenic, leading to an estimate of 25 μg/g creatinine (sum of the four metabolites) after 5-days of exposure. This prediction was used to support the ACGIH Biological Exposure Index published in 2000.[22]

BE values were proposed for general population exposure to benzene (Hays et al., 2012) using existing PBK models. A variety of US government risk assessment values (including the USEPA chronic reference concentration) were translated into corresponding benzene levels in

[17] https://cran.r-project.org/web/packages/httk/index.html.
[18] https://cefic-lri.org/toolbox/induschemfate/.
[19] https://echa.europa.eu/candidate-list-table/-/dislist/details/0b0236e1807dbdfe.
[20] https://echa.europa.eu/substance-information/-/substanceinfo/100.002.209.
[21] https://ec.europa.eu/social/main.jsp?catId=148&langId=en&intPageId=684.
[22] https://www.acgih.org/arsenic-and-its-inorganic-compounds/.

blood, assuming chronic steady state exposures. Numerous PBK models have been developed for benzene in rodents and humans. The authors deliberately chose the simplest PBK model (Brown et al., 1998) that was still consistent with available human TK data from controlled exposures. As the study focused on deriving BE values by estimating steady-state benzene concentrations in blood (and corresponding urine concentrations), the Brown et al., 1998 model, being the simplest yet consistent with the study's objectives, was utilized for BE derivation. In their derivation of BE values for benzene in blood, the authors classified the robustness of the TK data and model as relatively high because the model was parameterized based on data from several controlled human exposure studies. On the other hand, TK data and PBK models for BE values in urine were considered of low robustness because none of the available controlled human exposure studies measured urinary benzene concentrations, nor any of the models predicted urinary benzene excretion. For the derivation of BE values in blood, the relevance of the biomarker (blood benzene) was medium-rated as it is not the immediate toxicant. However it was considered more relevant than urinary benzene, which was also modelled.

4.1.2. Deriving external Health-Based GV from HBM-GV

PBK models can be used for external concentration reconstruction through reverse dosimetry for an established exposure scenario. In this approach, internal chemical-exposure measured in biomonitoring samples are converted via PBK modelling to the corresponding external exposure levels. This approach has been instrumental in the increasing use of human data from epidemiological studies, accompanied by biomarker data in food safety risk assessment. For instance, in EFSA studies, blood/serum concentrations related to health effects in humans were used to derive NOAEL or BMDL values. These values were then converted to external exposure levels and led to the identification of tolerable weekly intakes (TWI) for, e.g., dioxins and dioxin-like PCBs (EFSA Panel on Contaminants in the Food Chain et al., 2018) and perfluoroalkyl substances (PFAS) (EFSA Panel on Contaminants in the Food Chain et al., 2020).

In 2020, the Risk Assessment Committee of the European Chemicals Agency (ECHA RAC) used this approach in an opinion document on the occupational exposure limit (OEL) of lead (Pb).[23] An OEL for airborne lead was derived from lead BLV on the basis of an existing PBK model. The aim was to set an air concentration limit value that would not result in the exceedance of health based BLV even after repeated exposure. Reverse PBK modelling has been used also in food safety risk assessment for lead in food (EFSA Panel on Contaminants in the Food Chain, 2010).

4.1.3. Deriving external Health-Based GV from human external exposure – Response data

PBK modeling can be used to derive an external HBGV if no HBM-GV exist but human internal exposure – response data are available. The ECHA RAC used ATSDR (2012)[24] existing reverse dosimetry with PBK models for cadmium, which causes a risk for renal and bone effects. Although airborne OEL was based on local lung effects, PBK model was used to ensure that those air levels do not result in toxicologically relevant systemic exposure (or increased urinary Cd levels).[25]

4.1.4. Deriving HBM-GV from POD of animal data

Animal dose–response studies are used to establish a POD. The POD is the highest dose level in a dose–response curve at which no or low adverse effect is observed in the most sensitive and relevant species. The POD can be the NOAEL or, in the absence of NOAEL, the lowest dose that triggers the adverse effect (lowest-observed adverse effect level, LOAEL). Furthermore, the POD can be statistically determined as BMDL. In inhalation exposure, the POD can be no-observed adverse effect concentration (NOAEC), low-observed adverse effect concentration (LOAEC), or the benchmark dose concentration (BMCL). The severity and potency of the observed adverse effects are relevant parameters to consider when selecting a POD. In general, the first adverse effect that occurs at the lowest concentration determines which study will be used to derive a POD. The identified POD in a critical animal study can be converted into a human internal concentration of a biomarker. The POD is set as the dose in an animal PBK model and the extrapolation to a human PBK model leads to the prediction of a corresponding human biomarker concentration, which could be potentially used as HBM-GV. The US National Institute for Occupational Safety and Health (NIOSH) established a recommended exposure limit (REL) for diacetyl based on PBK modelling to extrapolate rodent benchmark concentration estimates to human exposures.[26] In another example, OELs were proposed for N-methylpyrrolidone based on PBK modelling to calculate human equivalent concentrations of animal-based POD values for developmental effects (Poet et al., 2016). A further illustration of this approach is seen in the EFSA recent evaluation of BPA. In this evaluation, EFSA used human toxicokinetic data, which facilitated the identification of the area under the curve (AUC) and calculation of the human equivalent dose factor. This data was instrumental in the animal to human extrapolation process for BPA risk assessment (EFSA Panel on Food Contact Materials et al., 2023).

In many instances, uncertainty factors are employed during the extrapolation process to account for inter-species differences and variability in human populations. Despite this approach, utilizing rodent data for predicting PODs in humans has inherent limitations. These include variations in metabolic pathways, differences in physiological parameters, and potential species-specific responses to a chemical. Additionally, the reliance on animal models may not fully capture certain human-specific susceptibilities. These caveats emphasize the need for careful consideration and interpretation when utilizing animal data to predict human PODs and subsequent HBM-GVs based on biomarker concentrations.

4.1.5. Deriving HBM-GVs from in vitro toxicity studies

In vitro dose–response studies can be used to establish a POD. In a human PBK model for a studied toxicant, the predicted concentration in blood or at the target organ is assumed to be equal to the *in vitro* determined POD (Rotroff et al., 2010; Wetmore et al., 2012). The human PBK model can predict the toxicant's concentrations in a biological medium (e.g., blood, urine) corresponding to the *in vitro* POD. The predicted concentrations in the biological matrix can then be used as HBM-GV. For example, *in vitro* toxicity data were combined with human PBK model to predict dose–response curves for developmental toxicity of glycol ethers (Louisse et al., 2010).

4.1.6. Extrapolating HBM-GVs to different populations, chemicals, and exposure scenarios

New HBM-GVs for other chemicals, populations, and exposures might be derived from established values by extrapolation with PBK modelling. For example, an HBM-GV for workers could be used to derive a guidance value for a vulnerable population such as pregnant workers (same exposure but different population) or for the general population (different exposure and different population). This seems to be a rather unexplored avenue for generating new HBM-GVs when no or few data are available. The HBM4EU programme established a HBM-GV for urinary cadmium (U-Cd) in the general population, and used a PBK model to derive "alert" values of U-Cd according to age (Lamkarkach et al., 2021).

[23] https://echa.europa.eu/documents/10162/ed7a37e4-1641-b147-aaac-fce4c3014037.

[24] https://www.atsdr.cdc.gov/toxprofiles/tp5.pdf.

[25] 20958724-bcdb-e18d-db23-48ded07496cf (europa.eu).

[26] Occupational Exposure to Diacetyl and 2,3-Pentanedione | NIOSH | CDC.

4.2. Exposure assessment

PBK modelling can improve the interpretation of HBM data by predicting interindividual variability in exposure. Some of the factors contributing to HBM interindividual variability are discussed below.

4.2.1. Extrapolating general HBM data to different populations

HBM values can be extrapolated to predict internal doses for specific populations, e.g., male vs female (Deepika et al., 2022), adult vs. child (Deepika et al., 2022), and foetus (Balhara et al., 2022) with use of PBK models. The extrapolated internal doses could then be linked to the corresponding external exposures for the specific populations, which then could be compared to HBGVs. Two examples are provided where EFSA used PBK models. In 2020, EFSA (2020) established a new TWI for the sum of four common PFAS in adults. This TWI was based on two human studies that showed an inverse association between serum levels of the four PFAS and vaccine antibody formation in 1-year-old, predominantly breastfed, and 5-year-old children. PFAS levels in mothers' milk not leading to serum levels in infants that may decrease their vaccination response were modelled. Using a similar approach, in 2012, EFSA derived a TWI based on a PBK model relating mercury concentration in mothers' hair (biomarker) and neurobehavioral impairment in children (EFSA Panel on Contaminants in the Food Chain, 2012).

4.2.2. Relative importance of different routes of exposure

HBM data for a chemical are the result of aggregate exposure to that chemical from all possible source and routes of exposure. TK data and PBK modelling could provide important information on the contribution of the different routes of exposure to HBM concentrations. For example, a PBK model derived using controlled human exposure studies for xylene was used to investigate the contribution of dermal absorption of xylene vapor when volunteers were exposed to the OEL but wearing an air-fed half-mask (Loizou et al., 1999). The model demonstrated that dermal absorption of vapors accounted for about 1.8 % of the total body burden. Although this contribution may appear modest, it should not be overlooked in situations where exposures to high airborne concentrations are mitigated by only using respiratory protection.

4.2.3. Facilitating the interpretation of mixture exposure

HBM data can also provide insight into co-exposure patterns resulting from exposure to multiple chemicals from various routes and sources and over time. Therefore, such data are particularly valuable for assessing potential risks from combined exposure to multiple chemicals. The role of PBK modelling in assessing mixture toxicology has been growing and widely used to investigate and address combined effects and potential interactions to human health from the simultaneous exposure to multiple chemicals. Desalegn and colleagues (Desalegn et al., 2019) reviewed state-of-the-art PBK models for chemical mixtures and to evaluate the applications of PBK modelling for mixtures with emphasis on their role in chemical risk assessment. Binary mixtures and volatile organic compounds accounted for two-thirds of the chemical mixtures identified. They reported that the most common modelled exposure route and species were inhalation and rats, respectively. Competitive inhibition was the most common type of interaction among the various types of mixtures, and usually becomes a concern at concentrations higher than environmental exposure levels. This leads to reduced biotransformation, that either means a decrease in the amount of toxic metabolite formation or an increase in toxic parent chemical accumulation. The consequence is either lower or higher toxicity compared to that estimated for the mixture based on the dose addition model. PBK modelling, therefore, can play a central role in predicting interactions in chemical mixture risk assessment (Desalegn et al., 2019). Recently, a mixture risk assessment was carried out based on the HBM analyses of 29 chemicals known to disrupt male reproductive health in 98 young Danish men (Kortenkamp et al., 2022). Risk quotients were calculated for each chemical included in the mixture risk assessment as the ratio between daily intakes and reference doses. Daily intakes were estimated for each study participant based on internal exposure data. Personalized hazard indices (i.e., the sum of the risk quotients for all studied chemicals for each participant) showed substantial exceedances of acceptable mixture exposures, with BPA and DEHP identified as drivers of mixture risks. Thus, HBM, with the analyses of the personalized hazard index, can open new doors to mixture risk assessment.

4.3. Risk characterisation

The ratios between the HBM data (typically 95th percentiles of measured levels) and the HBM-GV can be used to calculate risk characterization ratios (RCR). An RCR lower than 1 indicates that the risk associated with the exposure to the studied chemical is not expected to raise any specific health concerns. In a recent study, Meslin et al. (Meslin et al., 2022) developed an HBM-GV for bisphenol S (BPS) following the systematic methodology proposed within the HBM4EU project (Apel et al., 2020). They characterized the risk associated with exposure to BPS based on HBM data available for Europe calculating RCR. The results showed that RCRs exceeded 1 for BPS in the sampled populations.

4.4. Limitations

Integrating PBK modeling and HBM in risk assessment has several limitations, some of which have already been addressed here. These limitations include assumption of steady state conditions when calculating HBM-HVs. This assumption might not hold true for all chemicals, especially for those with shorter half-lives due to their rapid elimination, limited accumulation, and frequent fluctuations in body concentrations leading to underestimation of their actual exposure levels. The challenge of non-persistent chemicals with short half-lives is also the difficulty to measure internal exposure in human populations. Future work is needed to develop improved sampling strategies and biomarkers for non-persistent chemicals.

Another limitation is the translation of concentrations from accessible matrices like plasma or urine to specific target tissues. Current advancements in PBK modelling have improved our ability to estimate tissue concentrations. However, PBK models still necessitate research and validation to improve their accuracy in estimating target tissue levels. While PBK models can predict the time course of a chemical in the body and estimate internal exposure levels, more work is needed to enhance their reliability.

Additionally, there are uncertainties in reconstructing external exposure levels from biomarkers of exposure. This point that becomes particularly evident in the case of pesticides, where the same biomarker of exposure might originate from different parent compounds, complicating the accurate estimation of external exposure levels and the total margins of exposure for the associated parent compounds. This uncertainty is exacerbated by the HBM strategy of relying on single spot urine samples, which may not provide a reliable reconstruction of external exposure levels.

Lastly, there is a need for harmonized methods for deriving HBM-GVs across different regulatory silos. Efforts have been made to develop standardized protocols for deriving HBM-GV, but these need to be adopted and recognized by appropriate regulatory bodies.

5. Recommendations for the integration of HBM and toxicokinetics in human risk assessment

We provide seven major recommendations for the integration of HBM and TK in human risk assessment and explain below how these can be achieved.

5.1. Advancing HBM is important for the development of HBM-GVs and for direct assessment and interpretation of HBM data

Health-based HBM-GVs would strengthen decision-making for public and occupational risk assessments. Currently, there is no acknowledged harmonized method for deriving HBM-GVs across the different regulatory silos. Substantial efforts have been made by the HBM4EU initiative to develop standardized protocols for deriving HBM-GVs (Apel et al., 2020). However, these need to be adopted and recognized by appropriate regulatory and responsible bodies. The PARC project is working in this direction with the involvement of regulatory agencies (Marx-Stoelting et al., 2023). Additionally, the OECD (2022) has contributed to these efforts by publishing a guidance document for occupational biomonitoring and setting occupational biomonitoring limit values.

5.2. Combining HBM data with epidemiological and human toxicological data

This could better estimate the quantity of chemicals absorbed into the body, increasing the possibility of identifying an internal exposure–response relationship (Apel et al., 2020; Schütze et al., 2015), which can help guide public policies on chemical management (Choi et al., 2015). Regarding the design of HBM/epidemiological studies, it has been suggested that one of the most urgent needs is the setting up of a new generation of studies with improved and repeated biosample collection, improved questionnaire data, and the deployment of advanced exposure assessment methodologies, including HRMS-based methods as a first step to inform targeted methods (Vineis et al., 2020). While advocating for increased sample collection frequency for non-persistent chemicals (e.g., several times a day for urine and once a week for blood) over a long period of time (months or up to one year) may be ambitious, optimizing biosampling protocols to balance feasibility and relevance is crucial. Focused efforts to strategically schedule biosample collection considering chemicals' kinetics and half-life, while prioritizing key exposure periods or critical windows, could offer valuable insights into the toxicokinetics of chemicals and time-sensitive variations in chemical exposure without imposing unrealistic burdens on study participants.

5.3. Developing guidance and standardization for chemical analytical methods in HBM

These should describe biomarker selection and sampling methods based on human TK data as well as quality assurance (QA) and quality control (QC) for analytical chemical methods (Louro et al., 2019). An important outcome of HBM4EU was developing a QA/QC program for selected substances (López et al., 2021). Successful commercial QA programs that cover more chemicals than HBM4EU are available; however, they need to include all laboratories willing to participate in the QA/QC program as well as be further expanded to include other substances. Furthermore, to identify a greater number of biomarkers and more suitable ones, a suspect screening and full scan analysis should be performed instead of compound-specific methods. This effort will require a coordinated approach between experts in targeted and HRMS-based non-targeted/suspect screening approaches (Fig. 2). The use of innovative analytical methods based on high-resolution mass spectrometry (HRMS) should be encouraged as discovery-based approach because they provide comprehensive chemical fingerprints. With such an approach, targeted methods can be updated with the best metabolites to provide the most comprehensive view of the exposure, and the most appropriate biomarkers for the corresponding matrix. The current limitations of these HRMS-based methods are that accurate identification of new metabolites can be extremely time consuming and requires a lot of expertise, and that the synthesis of standards for individual chemicals and their metabolites are needed for accurate measurements. Highlighting ENTACT efforts in US (Ulrich et al., 2019) or similar initiatives serves as a testament to the collaborative efforts toward advancing analytical methodologies in HBM. Leveraging such collaborative initiatives can aid in addressing challenges, enhancing knowledge sharing, and fostering standardized practices, crucial for the successful implementation of HRMS-based methods in HBM research. HBM researchers sometimes need the assistance of manufacturers, perhaps 'encouraged' by regulators, to facilitate access to standard materials at 'reasonable' cost because metabolites are often not available and chemical synthesis of metabolites is generally prohibitive for an individual study.

5.4. Generating reliable TK data is key for PBK model parameterization, and for HBM optimization. Several recommendations can be made to this purpose

5.4.1 More efforts in validating new and existing *in vitro* models are

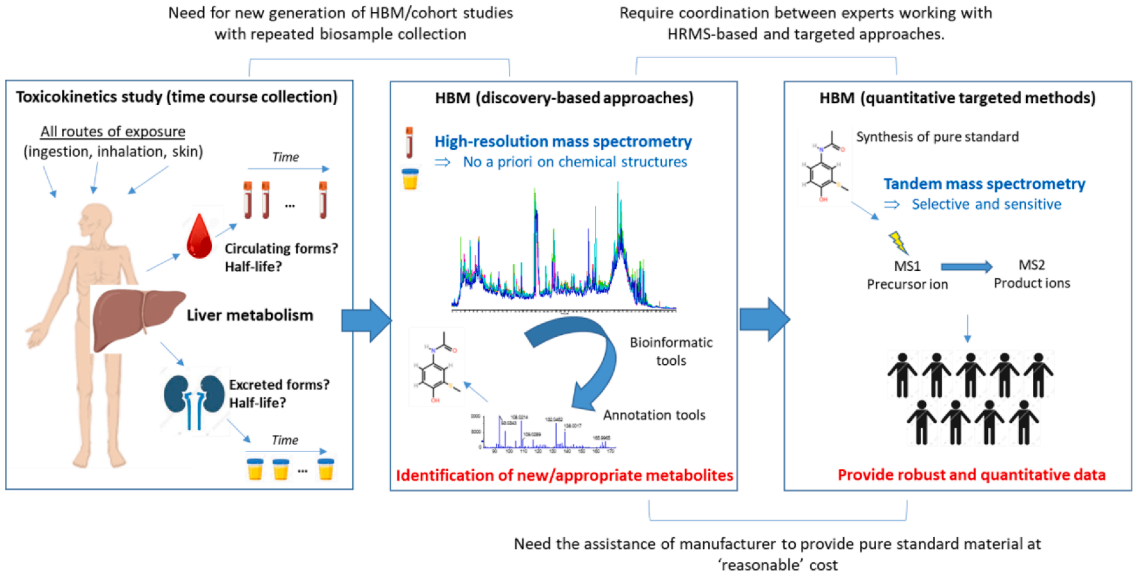

Fig. 2. Scheme of coordinated approach between toxicokinetics, high-resolution mass spectrometry, and MSMS experts to improve exposure assessment.

needed to generate ADME parameters and to reduce the variability of the parameter values which generate uncertainties in PBK model predictions (Louisse et al., 2020). The OECD has developed a guidance document (GD) on good *in vitro* method practices (OECD 2018a), and GDs and test guidelines (TG) to measure skin absorption (OECD 2004a; b) and hepatic intrinsic clearance using primary hepatocytes or S9 from rainbow trout (OECD 2018b; c; d). The OECD is currently developing a TG on the determination of CYP450 enzyme activity induction using differentiated human hepatic cells.[27] In addition to the officially accepted test methods, other *in vitro* and *in silico* methods for measuring ADME processes are available. A list of methods available to generate TK parameters with priorities set for the development of TGs has been proposed by Punt et al. (Punt et al., 2020).

5.4.2 Existing and new ADME databases should be publicly available (Bessems et al., 2014). These databases should contain at least the main parameters needed to parameterize simple PBK models (the permeability coefficient (Papp, cm/h) for passive crossing barriers (gut, skin, lung); distribution coefficients between air, blood, and tissues; protein binding in plasma; metabolic parameters such as Michaelis-Menten constant (Km, mmol/mL) and maximal rate (Vmax, mmol/h), or the intrinsic hepatic clearance (CLint, mL/h) for non-saturated metabolism; lung clearance, renal clearance).

5.4.3More regulatory initiatives should exist for generating TK data. Although regulatory initiatives are now advancing the move towards collecting TK data, its application to regulatory science has been limited. Except for pharmaceuticals and to a degree for plant protection products (Terry et al., 2016), toxicokinetic studies are generally not in the regulatory toxicological test requirements (e.g., REACH, CLP) (Louro et al., 2019). However, an OECD test guidelines on *in vivo* TK testing (OECD TG417) (OECD 2010) provides information on mass balance, absorption, bioavailability, tissue distribution, metabolism, excretion, and basic toxicokinetic parameters (OECD TG417 (OECD 2010)). The revised harmonized template (OECD Template, OHT 58[28]) highlights a number of important uses of TK data. While TK data are currently not present in standard data requirements for chemical risk assessment under EU REACH Regulation, the legislation states that the kinetic profile should be considered as part of the human health hazard assessment. The accompanying technical guidance outlines the potential role TK information can play in supporting intelligent testing strategies. This is a clear deficiency in regulatory schemes considering that TK studies could provide important information also for the identification of the target organ, for setting the appropriate experimental protocol in repeated exposure studies, and for reducing uncertainties in extrapolation from animal data to humans and facilitating animal-free toxicology. If TK is included in regulations, this could pave the way for a transition to more refined and accurate next generation chemical risk assessment. A pertinent example underscoring the importance of this recommendation is the most recent evaluation of glyphosate. Previously, risk assessment estimates based on urinary glyphosate concentrations were extrapolated to a daily intake value assuming a urinary excretion of 20 % of oral glyphosate excreted as unchanged glyphosate in urine (EFSA, 2015), with earlier estimates of 30 % (EC 2002). More recent human metabolism data has reported this rate as low as approximately 1 % (Faniband, 2020; Zoller et al., 2020). This was flagged in a recent study as having a significant impact on risk assessment approaches, as the oral glyphosate dose would be 20 times higher than previously assumed based on the same urinary glyphosate data, and demonstrated the diminished margin of safety for glyphosate exposures (Connolly et al., 2020). It was also highlighted that the urinary excretion rate would need to be embedded in future health-based reference values for glyphosate and in the development of future human biomonitoring guidance values (Connolly and Koch, 2023). The most recent EFSA evaluation of glyphosate took this into account, considering both oral absorption rates (i.e. 1 % and 20 %) and flagged the need to further consider ADME in regulatory risk assessment (European Food Safety Authority et al., 2023).

5.4.4 Validated PBK models should be used in a read-across approach for data-poor chemicals to predict TK data (Ellison, 2018; Ellison and Wu, 2020; Laroche et al., 2018; Paini et al., 2021; Thompson et al., 2021). The OECD PBK model guidance document (OECD, 2021) tries to provide guidance to address evaluation of PBK models for data poor chemicals for which no *in vivo* data are available for validation (Paini et al., 2021). Also, ECHA is now increasing the use of the read-across assessment framework (RAAF) because of the need to group substances for more effective regulatory action, reduce the need for safety testing, and prevent regrettable substitutions (ECHA, 2017). For many cases, TK data are considered valuable supporting evidence for read-across justification.

5.5. *Advancing the use of PBK modelling in risk assessment*

5.5.1 Increasing accessibility and user-friendliness by making existing PBK models publicly available with easy access to open source codes (e.g., the Open System Pharmacology suite (OSPS) publishes on Github all the developed code (Rostami-Hodjegan and Bois 2021)). The ISES Europe has recently published a PBK modelling inventory (Schlüter et al., 2022). Creation of a user-friendly open-source web based PBK model where risk assessors only need to impute a few parameters and the model would return predicted values could facilitate its use by risk assessors. Some examples are PK-Sim, IndusChemFate, and the EFSA TK-Plate (Quignot et al., 2018). The use of the FAIR (findable, accessible, interoperable, reusable) principles of scientific data sharing for *in silico* predictive models has been recently proposed (Cronin et al., 2023).

5.5.2 PBK models should be validated before risk assessors apply the models. The OECD has published a guidance document on the characterization, validation and reporting of PBK models for regulatory purposes (OECD, 2021).

5.5.3 Some criteria or qualifiers for PBK models should be communicated to guide risk assessors to choose the preferred model for a certain purpose. Examples are model applicability (scope), validation and uncertainty of parameters and predictions could be key for its utilization by risk assessors (Frechen and Rostami-Hodjegan, 2022).

5.5.4 PBK models should be used for aggregate exposure when dealing with risk assessment of a chemical coming from different sources and different exposure routes.

5.6. *Linking the aggregate exposure pathway (AEP) and the adverse outcome pathway (AOP) frameworks using PBK models*

This will improve the confidence in chemical and mixture risk assessment. Establishing relationships between exposure to a single chemical and a resulting adverse health outcome is the common practice in risk assessment nowadays. However, evaluation of risks for human health from the simultaneous exposure to multiple chemicals and disease outcomes is increasing, as reported in these overview documents (Beronius et al., 2020; Bopp et al. (2015)). As a result, there is a need to better understand the complex mechanisms that influence the journey of chemicals, from their initial environment release to the final biological effect they produce, which are all relevant for risk assessment. Just as the AOP framework has emerged as a means of providing insight into

[27] https://www.oecd.org/chemicalsafety/testing/Draft_TG_CYP_induction_for_2nd_WNT_review.pdf.

[28] https://www.bing.com/ck/a?!&&p=dcd892e6bf356963JmltdHM9MTY4MTc3NjAwMCZpZ3VpZD0wNTlkZjM3Ni1jM2E3LTZlYTQtMDFjMC1lMTlmYzI1NjZmNzImaW5zaWQ9NTE3Nw&ptn=3&hsh=3&fclid=059df376-c3a7-6ea4-01c0-e19fc2566f72&psq=OHT+58%2c+Endpoint+Study+Record%2c+Basic+Toxicokinetics&u=a1aHR0cHM6Ly93d3cub2VjZC5vcmcvZWhzL3RlbXBsYXRlcy9PSFQlMjA1OCUyMC0lMjBFTkRQT0lOVF9TVFVEWV9SRUNPUkQuQmFzaWNUb3hpY29raW5ldGljc192OS4xJTIwLU5vdiUyMDIwMjEuZG9jeA&ntb=1.

mechanism-based toxicity (Warner et al., 2022), the exposure science community has seen the recent introduction of the AEP framework (Tan et al., 2018; Teeguarden et al., 2016). An example was recently published for phthalates (Clewell et al., 2020) where an adaptable workflow for integrating exposure and toxicity data by coupling AEP and AOP frameworks and using *in vitro* and *in silico* methodologies for cumulative risk assessment is elucidated.

5.7. Integrating exposure biomarkers related to biologically effective dose into ADME studies and PBK modelling

Biomarkers of toxicologically effective dose may be considered as a very early stage of biomarkers of effect and flag that an exposure to a given chemical has reached a level sufficient to trigger a toxicologically relevant outcome through the interaction with molecular targets (Schmidt, 2006). Although they are not substance specific, they are triggered by chemicals and might be very useful in assessing overall exposures to chemical mixtures with additive effects. Four examples of effective dose biomarkers are: (1) changes in blood/serum enzyme activities (plasma and erythrocyte cholinesterases for organophosphate and carbamate pesticides)(Herrera-Moreno et al., 2021); (2) proteins induced downstream to receptor activation (eg., CYP1A1 for substances activating the aryl hydrocarbon receptor, including dioxins and PAHs) (Ho et al., 2022; Ibrahim et al., 2020); (3) metabolites and especially metabolite ratios (e.g., the sphinganine:sphingosine ratio as biomarker for the mycotoxin fumonisin) (Wangia et al., 2019); and (4) DNA adducts for carcinogenic substances and mixtures (Lu et al., 2021). These biomarkers are relevant to, or even coincide with, the molecular initiating events or subcellular key events of AOPs (e.g., for mycotoxins (Van Den Brand et al., 2022)), hence they flag health-relevant events.

6. Conclusion

A consistent and structured input on the main determinants that explain inter-individual variability into PBK modelling will support an evidence-based use of HBM. The use of internal exposure-based GVs by regulatory authorities could allow initial screening of population exposure to chemicals to identify those chemicals requiring more detailed exposure and risk assessment, assisting in priority setting for new policy action and ultimately leading to improved product stewardship and risk management (Boogaard et al., 2011). The main limitations to this approach in risk assessment are the lack of HBM guidance values, which require more TK information to support the interpretation of HBM data, and the lack of legal enforcement. In conclusion, the use of TK modelling in combination with HBM in risk assessment has high potential both in regard to single chemicals and mixtures.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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