



Consensus Report

New Approach Methodologies (NAMs) for Human-Relevant Biokinetics Predictions: Meeting the Paradigm Shift in Toxicology Towards an Animal-Free Chemical Risk Assessment

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Abstract

For almost fifteen years, the availability and regulatory acceptance of new approach methodologies (NAMs) to assess the absorption, distribution, metabolism and excretion (ADME/biokinetics) in chemical risk evaluations are a bottleneck. To enhance the field, a team of 24 experts from science, industry, and regulatory bodies, including new generation toxicologists, met at the Lorentz Centre in Leiden, The Netherlands. A range of possibilities for the use of NAMs for biokinetics in risk evaluations were formulated (for example to define species differences and human variation or to perform quantitative *in vitro-in vivo* extrapolations). To increase the regulatory use and acceptance of NAMs for biokinetics for these ADME considerations within risk evaluations, the development of test guidelines (protocols) and of overarching guidance documents is considered a critical step. To this end, a need for an expert group on biokinetics within the Organisation of Economic Cooperation and Development (OECD) to supervise this process was formulated. The workshop discussions revealed that method development is still required, particularly to adequately capture transporter mediated processes as well as to obtain cell models that reflect the physiology and kinetic characteristics of relevant organs. Developments in the fields of stem cells, organoids and organ-on-a-chip models provide promising tools to meet these research needs in the future.

1 Introduction

There are clear societal and scientific needs for the development and validation of predictive animal-free methods for safety evaluations to prevent adverse effects in humans caused by exposure

to chemicals (EU, 2010; OECD, 2018a; Oredsson et al., 2019). Whereas much work has been devoted to the development of *in vitro* screening methods to capture biological effects (toxicodynamics) of chemicals, insight into the absorption, distribution, metabolism and excretion (i.e., ADME/biokinetics) of chemi-

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cals also plays a central role in next-generation risk evaluations that move away from animal experimentation and towards animal-free methods (Albrecht et al., 2019; Bessems et al., 2014; Coecke et al., 2013; Desprez et al., 2018; Thomas et al., 2019; Wambaugh et al., 2018). These biokinetic data are needed for the adequate design of *in vitro* toxicity studies with respect to the application of physiologically relevant chemical test concentrations and inclusion of relevant metabolites for testing. In addition, biokinetic data play a crucial role in the process of quantitative *in vitro*-to-*in vivo* extrapolation (QIVIVE) of *in vitro* toxicity results to obtain equivalent oral/skin/inhalation human potency estimates that can be compared with human exposure to define the risk (Blaauboer, 2010; DeJongh et al., 1999; Louisse et al., 2017; Wambaugh et al., 2018; Yoon et al., 2012).

New approach methodologies (NAMs) for biokinetics include both *in vitro* approaches (experiments using preferably human tissue material or cells), computational (*in silico*) approaches, and combinations thereof. Examples of different *in vitro* methods for kinetics that can be performed with animal or human tissue material are: Transwell studies using Caco-2, MDCK or LLC-PK₁ monolayers for intestinal absorption and transporter studies, artificial human skin for skin absorption studies, metabolism models with (primary) human or animal (liver) cells or tissue fractions, plasma protein and tissue binding assays, and placenta transport experiments with human BeWo cells (Punt et al., 2017; Strikwold et al., 2017; Wilk-Zasadna et al., 2015). Recent research also focusses on the development of organ-on-a-chip models to improve the accuracy of individual methods by better physiological resemblance and to integrate the methods for different organs within one platform (Maschmeyer et al., 2015; McAleer et al., 2019; Prantil-Baun et al., 2018; Santbergen et al., 2019; van der Made et al., 2019).

Different types of computational (*in silico*) approaches for biokinetics can be distinguished. *In silico* approaches are first of all used for predictions of the behavior of a chemical in a body based on the structural properties of the chemical. Examples are quantitative structure activity relationships (QSARs) for absorption (Hou et al., 2004) and different prediction models for tissue partitioning (Berezhkovskiy, 2004; Poulin and Theil, 2002; Rodgers and Rowland, 2006). Secondly, computational approaches are very powerful for the integration of the various *in vitro* and *in silico* findings with either classical (one- or two-compartment models) or physiologically based kinetic (PBK)/physiologically based pharmacokinetic (PB(P)K) models (Bessems et al., 2014; Jamei, 2016; Louisse et al., 2017; Prantil-Baun et al., 2018; Punt, 2018). For example, *in vitro* methods using human cells or tissue fractions are generally used in combination with *in silico* calculated partition coefficients to parameterize PB(P)K models for predicting human biokinetics (Jamei, 2016, 2020; Jones and Rowland-Yeo, 2013; Wambaugh et al., 2018).

Despite the relevance of human- (fully animal-free) and animal-derived *in vitro* methods and the different *in silico* methods

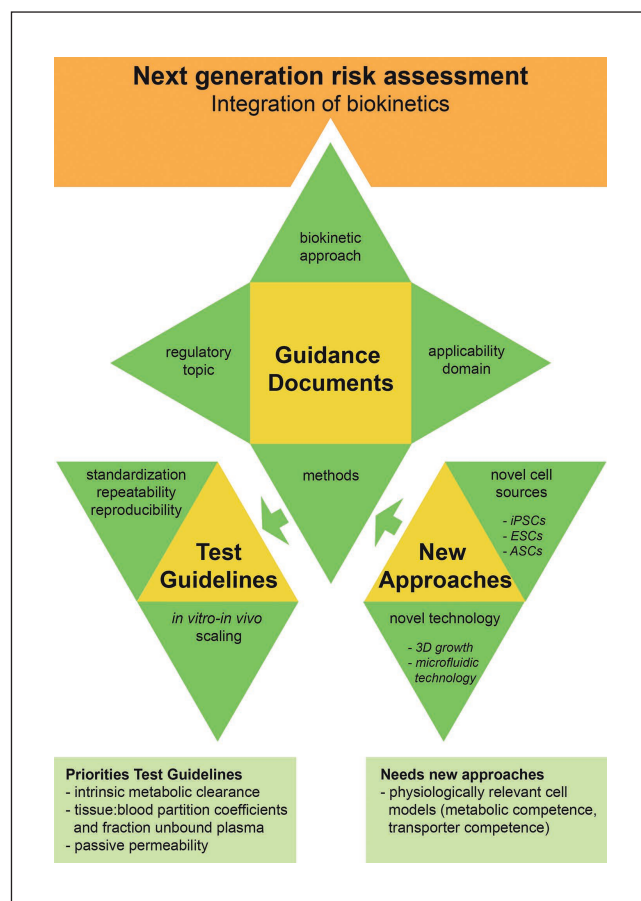


Fig. 1: Developments that are needed to increase regulatory use and acceptance of NAMs for biokinetics in risk evaluations and support a transition towards next generation risk evaluations that move away from animal experimentation

for biokinetics, the adoption of NAMs for biokinetic considerations in regulatory risk evaluations is lagging behind (Gellatly and Sewell, 2019; Punt, 2018; Tan et al., 2018; Zhuang and Lu, 2016). This challenge and the ambition of the Dutch government to become world leading with respect to animal-free research by 2025 formed the starting point for Wageningen Food Safety Research (WFSR) and the National Institute for Public Health and the Environment (RIVM) to organize a workshop on this topic at The Lorentz Centre in Leiden in The Netherlands in October 2017. The general goal of the Lorentz Centre is to grant and facilitate scientific discussions that can lead to ground-breaking changes in any specific field of research. A team of 24 experts from science, industry, and regulatory institutions, including new generation toxicologists, came together to define a strategy to move the field of biokinetics forward. *A priori* defined goals were (1) to

Abbreviations

ADME, absorption, distribution, metabolism and excretion; AOP, adverse outcome pathway; ASC, adult stem cell; ECHA, European Chemicals Agency; EFSA, European Food Safety Authority; ESC, embryonic stem cell; GD, guidance document (OECD); iPSC, induced pluripotent stem cell; KE, key event; KER, key event relationship; MIE, molecular initiating event; NAMs, new approach methodologies; OECD, Organisation for Economic Co-operation and Development; OECD WNT, OECD working group of national coordinators of the TGs programme; OHT, OECD harmonized template; PB(P)K, physiologically based (pharmacokinetic); qAOP, quantitative AOP; SCCS, Scientific Committee on Consumer Products; TG, test guideline (OECD)

make an inventory of ADME considerations within regulatory risk evaluations that can be addressed using NAMs for biokinetics in both current and next generation risk evaluations that move away from animal experimentation, (2) to define what is needed to increase regulatory use and acceptance of available innovative biokinetic approaches, and (3) to define opportunities for new scientific developments. Based on the discussions around these questions and an evaluation of the recent literature, recommendations (see summary Fig. 1) were made on the steps that are needed to achieve a better incorporation of alternative methods for biokinetics in regulatory chemical risk evaluations.

2 Opportunities for NAMs for biokinetics in current and next generation risk evaluations that move away from animal experimentation

As the field of toxicology is moving away from “black box” animal experimentation towards approaches to better understand the internal concentrations of compounds in relation to their mecha-

nisms of toxicity in humans (NRC, 2007; Thomas et al., 2017), the potential of NAMs for biokinetics is increasingly recognized by regulatory bodies (ECHA, 2014; EFSA, 2014; EMA, 2018; FDA, 2020). Formal incorporation of these approaches in regulatory toxicology (for, e.g., plant protection products, pharmaceuticals, chemicals or food additives) is, however, still limited (Gellatly and Sewell, 2019; Punt, 2018; Tan et al., 2018; Zhuang and Lu, 2016). To find means to increase the use of NAMs for biokinetics, an inventory was made of ADME considerations within regulatory risk evaluations that can be addressed with these approaches based on different EU regulations and guidelines in which the use of *in vitro* and *in silico* methods for kinetics methods is mentioned (Tab. 1, 2). Such an inventory is key to the future development of Organisation for Economic Co-operation and Development (OECD) guidelines. These can be guidance documents (GD) on how to use alternative methods to inform risk evaluations and test guidelines (TG) on how to perform a specific *in vitro* biokinetic study, which are generally combined with OECD harmonized templates (OHT; standard data formats for reporting) (OECD, 2018a).

Tab.1: Inventory of ADME considerations within EU regulatory risk evaluations that can be addressed using NAMs for biokinetics

ADME consideration	NAMs currently included in guidance documents/regulations	Are biokinetic data required?
Species differences <ul style="list-style-type: none"> – Are there differences in the profile of compound metabolites between the laboratory animals used in the toxicity study and humans? – Are there differences in metabolic rate constants (rate by which a chemical is converted into its metabolites)? 	Regulation (EU) No 283/2013 on the data requirements of active substances in plant protection products: “ <i>Comparative in vitro metabolism studies shall be performed on animal species to be used in pivotal studies and on human material (microsomes or intact cell systems) in order to determine the relevance of the toxicological animal data and to guide in the interpretation of findings and in further definition of the testing strategy.</i> ” (EC, 2013).	Yes. However, only the qualitative aspects of species differences in metabolism are evaluated (i.e., differences in types of metabolites), while quantitative differences in rates of metabolic conversion can be just as important.
	<ul style="list-style-type: none"> – Is the default uncertainty factor for species differences in kinetics appropriate in risk evaluations? – What is the difference in plasma/ tissue concentrations of a parent chemical/toxic metabolite between the laboratory animals of the toxicity study and humans (requires integration of <i>in vitro</i> data in kinetic models)? 	Mentioned in (e.g.): <ul style="list-style-type: none"> – SCCS Notes of Guidance for the Testing of Cosmetic Ingredients (SCCS, 2018). – ECHA endpoint specific guidance, including a fictional example of using PBK modelling and the development of assessment factors (ECHA, 2017b). – Guidance on the Biocidal Products Regulation Volume III Human Health-Assessment and Evaluation (Parts B+C) (ECHA, 2017a).
Special population, interindividual human variation, drug-drug interactions <ul style="list-style-type: none"> – What is the expected plasma/ tissue concentration of a drug or chemical in children? – Are there any subpopulations potentially at extra risk due to altered kinetics (e.g., ethnic-related polymorphism, as a result of lifestyle factors such as smoking or obesity, or related to diseases like chronic kidney failure)? 	EMA/CHMP/458101/2016 guideline on the reporting of physiologically based pharmacokinetic (PB(P)K) modelling and simulation: “ <i>Presently, the main purposes of PB(P)K models in regulatory submissions are to qualitatively and quantitatively predict drug-drug interactions (DDIs) and support initial dose selection in paediatric and first-in-human trials.</i> ” (EMA, 2018).	No, but increasingly included in regulatory dossiers (Jamei, 2016).



ADME consideration		NAMs currently included in guidance documents/regulations	Are biokinetic data required?
	Is the default uncertainty factor for human variation in kinetics appropriate in risk evaluations?	<ul style="list-style-type: none"> – Possibilities for PB(P)K modelling of human variation are mentioned in, e.g.: SCCS Notes of Guidance for the Testing of Cosmetic Ingredients (SCCS, 2018). – ECHA endpoint specific guidance, including a fictional example of using PBK modelling and the development of assessment factors (ECHA, 2017b). 	No
	Can any drug-drug interactions be expected?	EMA/CHMP/458101/2016 guideline on the reporting of physiologically based pharmacokinetic (PB(P)K) modelling and simulation: <i>“Presently, the main purposes of PB(P)K models in regulatory submissions are to qualitatively and quantitatively predict drug-drug interactions (DDIs) and support initial dose selection in paediatric and first-in-human trials.”</i> (EMA, 2018).	No, but increasingly included in regulatory dossiers (Jamei, 2016)
Read-across	Is the selected source chemical appropriate for read-across, having similar kinetics, including similar metabolites?	<p>Mentioned in, e.g.:</p> <ul style="list-style-type: none"> – Regulation (EC) No 1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH): Grouping of substances and read-across approach – an illustrative example. Part 1: Introductory Note (ECHA, 2013). – Guidance on the Biocidal Products Regulation Volume III Human Health Assessment and Evaluation (Parts B+C) (ECHA, 2017a). 	No, read-across is voluntary. In case of REACH, considering the possibility for read-across is mandatory. This drives a need for insights into toxicokinetics.
Route-to-route extrapolation	What is the dermal or inhalation equivalent exposure level of an oral No-Observed-Adverse-Effect Level?	<p>Mentioned in, e.g.:</p> <ul style="list-style-type: none"> – SCCS Notes of Guidance for the Testing of Cosmetic Ingredients (SCCS, 2018). – ECHA endpoint specific guidance, including a fictional example of using PBK modelling and the development of assessment factors (ECHA, 2017b). – Guidance on the Biocidal Products Regulation Volume III Human Health –Assessment and Evaluation (Parts B+C) (ECHA, 2017a). 	No
Internal exposure-based waiving	Is intestinal/skin/inhalation uptake limited, allowing to waive internal exposure?	<p>Mentioned in, e.g.:</p> <ul style="list-style-type: none"> – EFSA guidance for submission for food additive evaluations (EFSA, 2012). – Specific rules for adaptation provided for, e.g., reprotox tests in Annex IX of Regulation (EC) No 1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). 	No
	Selection of chemicals for further testing based on highest expected internal exposure.	<p>Mentioned in (e.g.):</p> <ul style="list-style-type: none"> – EFSA guidance on the data required for the risk assessment of flavorings to be used in or on foods (EFSA, 2010). 	No
Dose extrapolation	Extrapolation of effect levels in animal studies obtained at high doses to lower human exposures.	<p>Mentioned in (e.g.):</p> <ul style="list-style-type: none"> – SCCS Notes of Guidance for the Testing of Cosmetic Ingredients (SCCS, 2018). – ECHA endpoint specific guidance, including a fictional example of using PBK modelling and the development of assessment factors (ECHA, 2017b). – Guidance on the Biocidal Products Regulation Volume III Human Health Assessment and Evaluation (Parts B+C) (ECHA, 2017a). 	No

Tab. 2: Inventory of regulatory topics in next-generation risk evaluations for which insights into biokinetics (using NAMs) are relevant

Goal	Regulatory topics that can be addressed ^a
Guiding the design of <i>in vitro</i> toxicity studies	<ul style="list-style-type: none"> – What are physiologically relevant internal exposure conditions of the chemical in humans? – Which metabolites need to be taken into account in the <i>in vitro</i> studies? – Does the <i>in vitro</i> toxicity assay have the adequate metabolic competence? – Which tissues are relevant to include in the toxicity determination? – How does the chemical behave in an <i>in vitro</i> system with respect to protein, lipid and plastic binding, and in terms of evaporation? – Aiding the development of novel <i>in vitro</i> systems like organ-on-chip systems to determine dimensions and operating conditions.
Quantitative <i>in vitro</i> to <i>in vivo</i> extrapolations (QIVIVE)	<ul style="list-style-type: none"> – What are the internal levels of a chemical that are reached in a certain exposure scenario and how do these relate to <i>in vitro</i> effect concentrations (forward dosimetry approach)? – Translation of an <i>in vitro</i> biological effect concentration/exposure (e.g., EC10, BMC10) to an equivalent oral/skin/inhalation dose/exposure. – Improving the interpretation of human biomonitoring studies to reconstruct internal exposure levels for exposure assessment, QIVIVE and IVIVE-PBPK.

^a Topics related to human variation, route-to-route extrapolation, dose-extrapolations, read-across, and exposure-based waiving/priority setting, as described in Table 1 for current risk assessment procedures, remain of importance in next-generation risk evaluations.

2.1 Opportunities in current risk evaluations

Table 1 provides a summary of ADME considerations within regulatory risk evaluations that can be addressed with NAMs for biokinetics. This overview was obtained by exploring EU guidelines for chemical risk evaluations within different domains (e.g., pharmaceuticals, chemical substances, food additives, cosmetics) (ECHA, 2013, 2014, 2016, 2017a-c; EFSA, 2010, 2014; EMA, 2018; EU, 2010; SCCS, 2018).

Of the different *in vitro* and *in silico* approaches that are available for biokinetics, PB(P)K modelling is the most frequently mentioned tool. For example, in the field of pharmaceuticals, PB(P)K modelling plays a significant role to support initial dose selection in first-in-human or pediatric trials as well as in the prediction of drug-drug interactions (EMA, 2018; Jamei, 2016; Sato et al., 2017; Shebley et al., 2018; Taskar et al., 2019). PB(P)K models within this context are generally developed bottom-up, starting from *in vitro* and/or *in silico* kinetic data, after which the models are evaluated and/or fine-tuned against *in vivo* kinetic data. The label of several approved drugs already contains information on potential drug-drug interactions obtained by applying such PB(P)K modelling approaches (Jamei, 2016; Sato et al., 2017; Shebley et al., 2018; Taskar et al., 2019). Other documents, such as the guidance for testing of cosmetic ingredients by the European Scientific Committee on Consumer Safety (SCCS, 2018) and the guidance on information requirements and chemical safety assessment by the European Chemicals Agency (ECHA) (ECHA, 2014), refer to (bottom-up) PB(P)K modelling as well. In those cases, possibilities for the use of PB(P)K models are most frequently seen to predict species difference and/or human variation in plasma concentrations for refinement of the default inter/intra-species uncertainty factors for kinetics, or to perform dose or route-to-route extrapolations. However, so far, the actual use of PB(P)K modelling to meet these latter regulatory topics has been limited (Gellatly and Sewell, 2019; Punt et al., 2017; Tan et al., 2018).

Apart from PB(P)K modelling, stand-alone *in vitro* kinetic data (i.e., not integrated in a kinetic model) can also be requested

by regulatory authorities. A recent example is EC Regulation No 1107/2009, which requires the inclusion of *in vitro* metabolism studies (with microsomes or intact cell systems) in regulatory dossiers of plant protection active substances in order to determine the human relevance of the animal species chosen for the toxicological studies in a dossier. A recent EFSA workshop focused on the key elements to be considered for the interpretation of comparative *in vitro* metabolism studies and the minimum amount of information that must be obtained to satisfy the data requirement (EFSA, 2019).

Stand alone *in vitro/in silico* kinetic data may also be used for exposure-based waiving of toxicity tests. An example can be found in the EFSA “Guidance for submission for food additive evaluations” (EFSA, 2012). In this guideline, it is indicated that “a negligible metabolic conversion of an additive by gastrointestinal fluids or the gut microbiota (*in vitro*) and negligible absorption (*in vitro*), together with absence of genotoxicity, provides scientific justification for not undertaking higher-tier kinetic and toxicological studies.” Another example of the use of kinetic data for exposure-based waiving can be found within the European REACH legislation for industrial chemicals for some cases. Within REACH, there is no specific requirement to generate biokinetic data (ECHA, 2017b). For chemicals that are manufactured or imported in quantities of 10 tons or more, an assessment needs to be made of the biokinetic behavior of the substance to the extent that can be derived from the relevant available information (Annex IX Regulation (EC) No 1907/2006). These kinetic data, which may include data from NAMs, can be used to guide the design of appropriate toxicity studies or to waive studies. For example, no reproductive toxicity tests are required if a substance “is of low toxicological activity (no evidence of toxicity seen in any of the tests available), it can be proven from biokinetic data that no systemic absorption occurs via relevant routes of exposure (e.g., plasma/blood concentrations below detection limit using a sensitive method and absence of the substance and of metabolites of the substance in urine, bile or exhaled air) and there is no or



no significant human exposure” (Annex IX Regulation (EC) No 1907/2006). Given that all three requirements need to be met as a waiver for reproductive toxicity tests, the impact of the biokinetic data as such will remain limited in this context.

A final area in which NAMs for biokinetics are emerging is the justification of read-across. The principle of read-across is to predict endpoint information for a data-poor substance by using data from (an)other related substance(s) (Cronin and Yoon, 2019; Escher et al., 2019). The role of biokinetics in read-across becomes clear from different ECHA read-across guidelines for REACH as well as the biocidal product regulation (ECHA, 2013, 2017a, 2017b). According to these guidelines, the similarity in the biokinetics (e.g., type of metabolites and speed of metabolism) between the target and source substance(s) needs to be considered in the justification of the read-across approach (ECHA, 2013).

2.2 Opportunities in next generation risk evaluations that move away from animal experimentation

Compared with current risk evaluation procedures (Tab. 1), an even more important role of biokinetics is foreseen in next-generation risk evaluations that move away from animal experimentation (summarized in Tab. 2). Questions related to the extrapolation of animal data to humans are expected to become less important, but methodologies that incorporate human variation and allow route-to-route extrapolation, read-across and exposure-based waiving/priority-setting without the use of experimental animals will remain and may even become more important.

In addition, new applications of biokinetics will emerge. For example, NAMs for biokinetics will play a crucial role in the design of *in vitro* toxicity experiments. Particularly, given that not all *in vitro* biological effect assays include metabolism, it is important to characterize the metabolites that are formed in the human body and, if possible, to subsequently assess the potential toxic effects of these metabolites (Escher et al., 2019; Wilk-Zasadna et al., 2015). In addition, biokinetic information provides insights into whether specific exposure scenarios, such as irregular peak exposure concentrations, may lead to different cellular responses than continuous exposure to lower dose levels. In some cases there can also be a significant time delay between *in vitro* medium concentrations and the internal cell concentration (McAleer et al., 2019; Rozman and Doull, 2000). Such temporal concentration effects can be important to consider in the design of an *in vitro* toxicodynamic experiment and the subsequent translation of the results to actual human exposure scenarios. Finally, PB(P)K modelling can also be used as a tool to guide the design of *in vitro* experiments, for example by providing insight into dimensions and operating conditions for organ-on-a-chip systems (Abaci and Shuler, 2015; Hartung et al., 2017; Sung et al., 2014).

Another new role of biokinetics (and particularly PB(P)K modelling) in next-generation risk evaluations is to put *in vitro* biological activity data in the context of human exposure, also called QIVIVE (quantitative *in vitro* to *in vivo* extrapolations). This can be done based on a forward dosimetry approach (i.e., comparing predicted or observed internal plasma or tissue levels with the *in vitro* concentration-response curves) or based on a reverse dosimetry approach (i.e., extrapolating the *in vitro* effect concen-

trations to equivalent oral exposures) (Dancik et al., 2013; Rostami-Hodjegan, 2012; Yoon et al., 2012). An example of forward dosimetry is the comparison of an internal exposure with *in vitro* biological effect concentrations in so-called exposure-to-activity ratios (EARs) (Becker et al., 2014; Dent et al., 2019). This approach provides predictions on whether certain *in vitro* biological effects are likely to be induced in a certain exposure scenario and provides a means to prioritize the key biological targets, focusing on those that are elicited at exposures (concentration, duration, frequency, delay) that are relevant for the expected internal levels. Furthermore, EARs of different compounds can be compared to place the exposure-activity data of a chemical relative to a known reference compound (Becker et al., 2014; Dent et al., 2019). In contrast to forward dosimetry, reverse dosimetry approaches focus on the extrapolation of *in vitro* effective or benchmark concentrations (e.g., EC10, BMC10) into equivalent *in vivo* exposure-response curves (Louisse et al., 2017; Wetmore, 2015; Yoon et al., 2012). Kinetic modelling (including PB(P)K modelling) plays an important role in obtaining the required insights into the relation between external and internal exposure for QIVIVE, based either on forward or reverse dosimetry. A prerequisite within the context of animal-free testing strategies is that these models are developed based on *in vitro* and/or *in silico* input data themselves (Rostami-Hodjegan, 2012). Furthermore, good PB(P)K modelling practice as well as the quantification of uncertainty and variability within PB(P)K is of importance to gain confidence in the predictions and to estimate the credible interval around the predicted external dose levels that are linked to the *in vitro* effect concentrations (Jamei et al., 2009a; McNally et al., 2018; Pains et al., 2019; Wambaugh et al., 2019). Alternatively to kinetic (computer) modelling, exposure measurements from human biomonitoring studies (blood, urine, or milk) could be used for QIVIVE, though only for chemicals that are already marketed (Becker et al., 2014). Also then, PB(P)K modelling can be used for the interpretation of the human biomonitoring data, for example to translate a urinary concentration back to the corresponding blood concentration (Clewell et al., 2008; McNally et al., 2012, 2019; Rostami-Hodjegan, 2012).

A final role for biokinetics in next-generation risk evaluations is to establish freely available concentrations in *in vitro* assays. Irrespective of whether the internal predicted exposure levels are compared with the *in vitro* biological activity data based on forward dosimetry or based on reverse dosimetry, it is important to account for potential differences between the free concentration of the chemical in plasma (or the relevant organ or even cell under consideration) vs. the chemical in the *in vitro* toxicity test. Apart from protein and lipid binding, the free concentration of a chemical in an *in vitro* system can also be impacted by evaporation and/or binding to plastic or filter material (e.g., microtiter plates), reviewed in Kramer et al. (2015). Calculation tools to predict the free available concentration in an *in vitro* experiment (Armitage et al., 2014; Fischer et al., 2017; Fisher et al., 2019; Groothuis et al., 2015; Kramer, 2010; Zaldivar Comenges et al., 2017) and the fraction unbound in plasma (Lobell and Sivarajah, 2003; Yamazaki and Kanaoka, 2004) will therefore become crucial tools in next-generation risk evaluations.



3 Increasing the regulatory acceptance and use

Though the possibilities for NAMs for biokinetics are manifold (see above evaluation), formal inclusion is still limited due, amongst others, to the lack of robust and reproducible methods (Punt et al., 2017; Sewell et al., 2017; Tan et al., 2018; Zhuang and Lu, 2016). To this end, the experts at the workshop recommended to set up a continuous OECD expert group on biokinetics, analogous to the expert groups on, e.g., genotoxicity and skin sensitization, to stimulate and guide the generation of TGs for methods to generate biokinetic parameters in a robust and reproducible way and of GDs for their application in risk assessment procedures (see summary Fig. 1). In the meantime, this working group has been formally established. Within the context of the OECD, a GD focuses on how experimental results can be used to inform risk evaluations (i.e., to answer a specific regulatory question), and a TG focuses on how to perform a specific study (OECD, 2009). In the area of biokinetics, currently OECD TG 417 (with relative harmonized template OHT58) describes *in vivo* studies, providing the protocol for the conduct of studies on mass balance, absorption, bioavailability, tissue distribution, metabolism, excretion, and basic kinetic parameters (e.g., C_{max} , AUC) (OECD, 2010).

3.1 Standardization and development of TGs

The experimental conditions of *in vitro* kinetic studies can have a significant impact on the kinetic parameters that are obtained (e.g., metabolic clearance, absorption rates). This leads to a high variability in the estimates for these parameters for the same chemical among different studies as recently shown for metabolic clearance experiments (Louisse et al., 2020; Ring et al., 2011). One currently needs to evaluate each study in detail to find out whether the results are in agreement with what is expected *in vivo* and reliable enough to use for, e.g., PB(P)K modelling (e.g., is the intrinsic clearance measured at concentrations below the K_m , are the Michaelis-Menten kinetics (K_m and V_{max}) obtained at incubation conditions in which not too much of the substrate is depleted, which solvents were used as vehicle and at which concentrations, are the right cofactors added, and how was the data processed?). The development of standardized methods and TGs is needed to increase quality and transparency in the biokinetic results, to reduce inter-laboratory difference, and to derive kinetic parameters with a high *in vivo* (human) relevance. Such TGs should include information on the principle of the test, the experimental set-up, the inclusion of reference compounds, the calculation of the *in vitro* results (correction factors applied or modeling of the *in vitro* data), and the reporting of the results (Choi et al., 2019; Fisher et al., 2019; Jamei et al., 2009b). Recently, an OECD GD on good *in vitro* method practices (GIVIMP) for the development and implementation of *in vitro* methods for regulatory use in human safety assessment was published. GIVIMP addresses key aspects of good *in vitro* practice, including a clear definition of roles and responsibilities, procedures for storing and handling cells and tissues, ways to prepare test items and avoid cross-contamination, defining and describing standard operating procedures, and how to properly report results (OECD, 2018a). When developing TGs for NAMs for biokinetics, it is important

to take the GIVIMP guidance into account with respect to the critical aspects that need to be considered in the design of *in vitro* kinetic experiments in general. For regulatory use, the TGs should furthermore provide specific boundaries (test concentrations, time-point, etc.) within which experiments need to be performed to obtain results that are relevant to the human situation.

3.2 Development of GDs

Providing that the kinetic data are obtained with protocols that lead to robust and reproducible results, an important second step is to develop GDs that aid to systematically interpret submitted kinetic data (e.g., *in vitro-in vivo* scaling, defining uncertainties) and to define acceptance criteria (e.g., biological and technical considerations like maximum incubation times and acceptable concentration ranges). An example of an OECD GD in the field of biokinetics is the recently published OECD GD on the determination of *in vitro* intrinsic clearance using primary hepatocytes or S9 from rainbow trout and extrapolation hereof to an *in vivo* intrinsic clearance (OECD, 2018b). This GD provides information on the selection of the *in vitro* test system (S9 or primary hepatocytes), consideration of properties of the test compound (solubility), the inclusion of positive and negative controls, and how to use the *in vitro* data to meet the regulatory topic to improve predictions of chemical bioaccumulation in fish (OECD, 2018b). Similar GDs will be needed for the regulatory topics of human risk assessment that are included in Tables 1 and 2. However, it is recognized that for many of these questions the development of a GD may not be straightforward.

The complexity of developing a GD can be illustrated by taking the evaluation of species differences as an example. Although the evaluation of species differences can be expected to be phased out in next-generation risk evaluations, the development of GDs for this regulatory topic will still be crucial in the transition towards NAMs to gain confidence in outcomes by making use of available *in vivo* data for evaluation of the results. An approach to define species differences in kinetics based on *in vivo* data is for example to compare measured plasma concentrations and/or AUCs of a toxic agent (parent compound or metabolite) between experimental animals and humans (corrected for differences in, e.g., dosing and protein binding) (Meek et al., 2003). To obtain such a comparison with NAMs is not straightforward as there can be various causes for differences in plasma concentrations. Interspecies differences in metabolism are the best recognized of these (Cao et al., 2006; Musther et al., 2014). Many metabolizing enzymes, such as cytochromes P450 (Martinez et al., 2019; Nishimuta et al., 2013), UGT (Chiu and Huskey, 1998; Deguchi et al., 2011) and SULT (Punt et al., 2007; Wang et al., 2009) are differently expressed/active in rat, dog, monkey and mouse compared to humans. By measuring metabolic conversions with primary hepatocytes or tissue fractions, insights into species differences in metabolic rates and profiles can be obtained. However, focusing on species differences in liver metabolism may overlook other potential kinetic processes that influence plasma concentrations. Expression and/or activity of intestinal transporters, for example, can also differ between humans and animals. While rodents for instance express Mdr1a and Mdr1b transporters, hu-



mans only express one MDR1-encoded protein (P-glycoprotein, P-gp), and BCRP expression is high in rodent kidneys but low in humans (Chu et al., 2013). Differences in physiology may need to be accounted for as well when predicting absorption on the basis of NAMs, including differences in the small intestinal transit time (Sutton, 2004), the intestinal pH and radius (Dressman and Yamada, 1991), microbiome composition (Behr, 2019), and differences in the structure of the gastro-intestinal tract, e.g., no circular folds in the dog (Slatter, 2003) that affect the surface area. Differences in distribution in rat, mouse, dog and human have been associated with differences in protein binding and active transport into organs (Grover and Benet, 2009). In case of renal clearance, species differences can range from 1.6- to 13-fold as a result of differences in passive renal clearance alone (Walton et al., 2004). Ideally, all these kinetic processes are integrated in a PB(P)K model to allow evaluation of species differences on plasma concentrations of the chemical (Musther et al., 2017). Though PB(P)K models can be made as complex as needed, in practice a balance must be found between accuracy (and therefore complexity) and simplicity (ease of use) (Bois, 2010). This also needs to be kept in mind for the development of GDs. Rather than directly focusing on covering all sources of species differences in kinetics, it will be important first to enhance the use of individual (human-based) NAMs for kinetics and to make sure that these are sufficiently understood and provide robust results for application in a regulatory context. In case of hepatic clearance studies, for example, the European Union Reference Laboratory for alternatives to animal testing (EURL ECVAM) recently established a systematic framework to characterize *in vitro* methods for human hepatic metabolic clearance in terms of their design, applicability and performance (Gouliarmou et al., 2018). In addition, guidelines are needed on the integration of *in vitro* kinetic data into *in silico* kinetic models (Paini et al., 2019).

4 Availability of NAMs for human-relevant biokinetics and the opportunities of new scientific developments

4.1 Methods that are considered ready for the development of TGs

In Table 3, priorities are set for the methods available for different kinetic processes for standardization and the development of TGs and research needs. Particularly, the generation of standardized approaches and a TG for hepatic metabolic clearance with primary hepatocytes or hepatic microsomes/S9 is considered a high priority. Gouliarmou et al. (2018) already made a start by establishing a systematic framework to characterize *in vitro* methods for human hepatic metabolic clearance to provide a basis for harmonization within the OECD. A TG for hepatic clearance can potentially be developed further into TGs for metabolite identification and extra-hepatic (i.e., intestine, kidney, lung, skin) clearance.

The development of TGs for different *in vitro* and/or *in silico* approaches that predict partition coefficients, fraction unbound in plasma, blood-plasma ratio, and *in vitro* biokinetics (free fraction as a result of protein binding, plastic binding or evaporation) is also considered relevant. Based on ample experience with the use of these *in silico* approaches in kinetic model development and

QIVIVE (Armitage et al., 2014; Fischer et al., 2017; Fisher et al., 2019; Kramer, 2010; Zaldivar Comenges et al., 2017), guidance on their use and applicability domain effectively can be developed. A final priority can be given to developing a technical guideline for Caco-2 (MDCK, LLC-PK₁, or PAMPA) absorption studies to measure passive intestinal permeability. Though alternatives to Caco-2 permeability experiments (co-cultures, 3D, medium flow, peristaltic stretching) have been developed (Costa and Ahluwalia, 2019), Caco-2 transwell studies remain the best established among different protocols for permeability measurement (Hubatsch et al., 2007) and can serve as a starting point for technical guidance development. Opportunities for the development of TGs for lung absorption experiments and transporter studies (intestine, kidney, lungs) are currently considered to be limited as further scientific research is needed to identify physiologically relevant cells and define scaling methods (see Section 4.2). An OECD TG already exists for *in vitro* dermal absorption studies (OECD, 2004), and a TG on measuring induction and inhibition processes of metabolic enzymes is currently being developed (Bernasconi et al., 2019; OECD, 2019).

4.2 Opportunities for new scientific developments

Whereas much can be achieved to increase regulatory acceptance and use of NAMs for biokinetics by standardization and the development of guidelines, this is currently not possible for all aspects of biokinetics. Especially capturing transporter-mediated absorption (intestine), distribution (e.g., blood-brain barrier, placenta, organ uptake) or excretion (e.g., active renal excretion, biliary secretion) with NAMs is still difficult (Clerbaux et al., 2018, 2019; Taskar et al., 2019). Clerbaux et al. (2018) performed a survey among seventy-three experts in the field of transporter kinetics to evaluate the applicability of transporter data for ADME considerations in regulatory risk evaluations. Respondents highlighted the complex interplay with metabolic enzymes and other transporters, species differences, lack of specific transporter substrates and inhibitors, loss of cell polarity, lack of negative controls, problems of inter-laboratory variability, and lack of standardized protocols as key challenges of transporter models (Clerbaux et al., 2018, 2019). For the use of transporter data in PB(P)K models, scaling approaches also are needed to account for the quantitative difference of transporters in cell lines versus tissues (Clerbaux et al., 2018; Galetin et al., 2017; Taskar et al., 2019).

Apart from the specific challenges with transporter kinetics, there is also a general need for new cell models to overcome many of the hurdles of the classically used tumor-derived cell lines as well as primary cell cultures. Widely used tumor-derived cell lines (e.g., Caco-2 intestinal cells, HepaRG or HepG2 liver cells and A549 lung cells) all have shortcomings to some extent. These either relate to the expression of metabolic enzymes and transporters or to the barrier properties (e.g., the ability to grow to confluence, polarized cell layer(s), mucus secretion) (Ehrhardt et al., 2017; Van Breemen and Li, 2005; Wilk-Zasadna et al., 2015). These shortcomings affect not only kinetic studies (measurements of metabolism or permeability rates) but also *in vitro* toxicodynamic studies that require, for example, metabolic activation. In contrast to tumor-derived cell lines, primary cells closely mimic the physiological state of cells *in vivo* but can on-



Tab. 3: Overview of the current availability of NAMs for different kinetic parameters and research needs

Kinetic processes	Research needs	Priority for OECD TG development	Related regulatory topic (see Tab. 1, 2 and references therein) ^a
Liver clearance	<ul style="list-style-type: none">– Standardization of incubation methods with (cryopreserved) primary human hepatocytes or S9/microsomes.– Evaluation of the applicability of non-primary cells (e.g., HepaRG™ cells).	Hepatic metabolic clearance studies with (cryopreserved) primary human hepatocytes or S9/microsomes are considered a high priority for the development of a TG. Well-established protocols are available. Hepatic clearance data are relevant to a large range of regulatory topics (see Tab. 1, 2) as a major determinant of blood and tissue concentrations (Cao et al., 2006; Musther et al., 2014). Potential for extension of the TG to HepaRG clearance studies as an alternative to primary cells, which are of limited availability.	<ul style="list-style-type: none">– Species differences– Human variation– Route-to-route extrapolations– QIVIVE
Metabolite identification	<ul style="list-style-type: none">– Standardization of methods that are used based on incubations with S9/microsomes/ (cryopreserved) primary hepatocytes.– Higher sensitivity and standardization of analytical chemical techniques and improved methods to characterize metabolites.– Applicability of non-primary cell cultures (e.g., HepaRG™).	TGs for comparative metabolite studies with S9/microsomes/ (cryopreserved) primary hepatocytes are considered a high priority, particularly as comparative metabolite studies are a data requirement within the EU regulation on plant protection products (EC, 2013). Potential for extension of the TG to HepaRG clearance studies as an alternative to primary cells, which are of limited availability.	<ul style="list-style-type: none">– Species differences in type of metabolites (required for plant protection products)– Read-across– Guiding <i>in vitro</i> toxicity test design– Identification of toxic agent
Extrahepatic metabolic clearance and metabolite formation	<ul style="list-style-type: none">– Show proof of principle with S9/microsomes/ (cryopreserved) primary cell cultures of extrahepatic tissues (with a focus on kidney, lung, intestine and skin).– Development of non-primary cell cultures with physiologically relevant metabolic capacity.– Contribution of the gut microbiota to the metabolic clearance of chemicals.	When TGs for hepatic metabolic clearance and metabolic formation are established, these can serve as a template for clearance measurements with S9/microsomes/ (cryopreserved) primary cell cultures of extrahepatic tissues (with a focus on kidney, lung, intestine and skin).	<ul style="list-style-type: none">– Species differences– Human variation– Route-to-route extrapolations– QIVIVE– Guiding <i>in vitro</i> toxicity test design– Identification of toxic agent
(Passive) intestinal absorption	<ul style="list-style-type: none">– Standardization of transwell absorption experiments (e.g., Caco-2).– Exploring value of added complexity (co-cultures, 3D, medium flow, peristaltic stretching).	Although protocols for Caco-2 transwell absorption studies are well established (Hubatsch et al., 2007), the applicability domain of a TG for these studies will be limited to passive absorption as challenges still exist in the adequate scaling of transporter-mediated influx/efflux processes (Clerbaux et al., 2018).	<ul style="list-style-type: none">– Route-to-route extrapolations– Exposure based waiving and priority setting– QIVIVE– Guiding <i>in vitro</i> toxicity test design
Tissue binding (fraction unbound in plasma, partition coefficients)	<ul style="list-style-type: none">– Standardization and the development of guidance documents of available <i>in vitro</i> and/or <i>in silico</i> methods.	Well-established <i>in vitro</i> methods and <i>in silico</i> calculation tools (Peters, 2012) can form the basis for the development of TGs.	<ul style="list-style-type: none">– Species differences– QIVIVE– Guiding <i>in vitro</i> toxicity test design

^a The mentioned *in vitro* and *in silico* methods for different kinetic processes should be considered as providing parts of the information that is needed to answer the mentioned regulatory topics. An integrated approach (combined *in vitro* and *in silico* approaches) will be needed to cover the regulatory topics as a whole.



Kinetic processes	Research needs	Priority for OECD TG development	Related regulatory topic (see Tab. 1, 2 and references therein) ^a
<i>In vitro</i> biokinetics (fate of a compound in an <i>in vitro</i> assay)	<ul style="list-style-type: none"> Standardization and development of guidance documents for available calculators based on physicochemical properties 	Various <i>in silico</i> tools are available for animals and humans with different degrees of complexity but outcomes may vary depending on the applicability domain (Groothuis et al., 2015).	<ul style="list-style-type: none"> QIVIVE Guiding <i>in vitro</i> toxicity test design
(Passive) lung absorption	<ul style="list-style-type: none"> Development of non-primary cell cultures with physiologically relevant transporter activity and metabolic capacity. 	Availability of <i>in vitro</i> tools is still limited. Calu-3 monolayer assays are currently the most promising for regulatory use (Ong et al., 2013).	<ul style="list-style-type: none"> Route-to-route extrapolations Exposure based waiving and priority setting QIVIVE Guiding <i>in vitro</i> toxicity test design
Transporter mediated absorption or excretion processes	<ul style="list-style-type: none"> Development of <i>in vitro</i> methods that contain physiologically relevant transporter activity (required for intestine, kidney, liver, placenta, blood-brain barrier). Development of adequate scaling methods. 	Low priority due to limitations in currently available methods as described in Section 4.2 (Clerbaux et al., 2018, 2019), current capabilities and challenges are highlighted in a recent review (Taskar et al., 2019).	<ul style="list-style-type: none"> Species differences Human variation Route-to-route extrapolations QIVIVE Guiding <i>in vitro</i> toxicity test design
Induction and inhibition processes of metabolic enzymes		Currently being developed (Bernasconi et al., 2019; OECD, 2019b). CYP induction/inhibition can be seen as biomarker for metabolic competence of cells and as potential mechanism of action.	<ul style="list-style-type: none"> Human variation, including drug-drug interactions Species differences
Dermal absorption		OECD TG 428 already exists.	<ul style="list-style-type: none"> Route-to-route extrapolations QIVIVE Guiding <i>in vitro</i> toxicity test design

ly be kept in culture for a limited time. Hence, developments are needed to overcome these different challenges.

New technologies involving telomerase overexpression in primary cells, induced pluripotent stem cells (iPSCs), embryonic stem cells (ESCs), or adult stem cells (ASCs) as well as new culturing techniques like 3D spheroid or organoid cultures and organ-on-a-chip technology are expected to provide novel solutions in the field of NAMs for biokinetics.

Relevant examples include the following developments:

- a non-cancerous human renal proximal tubular cell line, RPTEC/TERT1, that has been utilized successfully for several long-term kinetic studies of cyclosporine A, cisplatin and adenofovir, showing promising results due to the expression of many relevant transporters and metabolizing enzymes (Aschauer et al., 2015; Wilmes et al., 2015),
- bioengineered kidney tubule, intestinal tubule and bile ducts obtained by culturing immortalized cell lines or ASCs on collagen-coated hollow fiber (Chen et al., 2018; Faria et al., 2019; Jochems et al., 2019),
- alternatives to Caco-2 cells, such as intestinal organoids from iPSCs, differentiated in the presence of small-molecule com-

pounds to increase the expression of intestinal markers and pharmacokinetic-related genes (Onozato et al., 2018),

- HepaRG cells as a metabolically competent tumor cell line that provides promising results with respect to intrinsic hepatic clearance measurements (Zanelli et al., 2012) and metabolic activation of chemicals in toxicity studies (Louisse et al., 2019),
- spheroids from primary hepatocytes or HepaRG cells that express relevant bile acid transporters (Hendriks et al., 2016),
- three-dimensional cultures from primary alveolar cells (Huang and Hsu, 2014),
- multi-organ platforms (fluidic coupling of different organs-on-a-chip) that allow, for example, to mimic the effect of liver metabolic activation/detoxification on other target organs (e.g., McAleer et al., 2019; Wang et al., 2018).
- the combination of cells or organoids derived from iPSCs, ESCs or ASCs in microfluidic systems, showing that particularly the combination of novel cell sources with new culturing techniques may improve the metabolic and transporter competence of cells *in vitro* as a result of shear stress (e.g., Homan et al., 2019; Starokozhko et al., 2018).

Although these examples show that *in vitro* approaches that better reflect human physiology are emerging fast, the applicability of these approaches to obtain kinetic parameters still needs to be explored (see summary Fig. 1).

5 Integration of NAMs for biokinetics in a next-generation risk assessment framework

New developments in animal-free methods for biokinetics should not stand by themselves. Efforts are needed to seamlessly connect different approaches within next-generation risk assessment, particularly to link the biokinetics and the adverse effects (toxicodynamics) of a chemical. With next generation risk assessment frameworks, methods like high-throughput transcriptomic and high-content imaging followed by more specific assays play a crucial role in the screening of chemicals for biological effects (Thomas et al., 2019). As long as the results of these assays provide quantitative information (e.g., concentration/exposure response curves), kinetic data can be linked to these biological effect assays to obtain equivalent oral/skin/inhalation human potency estimates that can be compared with human exposure estimates to define the risk.

For the use of *in vitro* biological effect data and QIVIVE, *in vitro* toxicity studies should be able to pick up all relevant interactions with biological targets or pathways. The adverse outcome pathway (AOP) framework currently provides the most effective framework to organize and describe biological effects in terms of molecular initiating events (MIEs), key events (KEs), and key event relationships (KERs) in relation to an adverse outcome (Leist et al., 2017; Vinken et al., 2017). At present, a direct link between biokinetics (including QIVIVE) and AOPs is not straightforward. Consideration of biokinetics is not incorporated directly into an AOP description, particularly given that AOPs are not chemical-specific. In addition, AOPs are currently mainly qualitative in nature, providing (narrative) descriptions of the KERs, i.e., the relation between MIEs, KEs, and an adverse outcome (Edwards et al., 2015; Vinken, 2018). Though qualitative AOPs are relevant for hazard identification, moving towards the use of AOPs in risk evaluations also requires quantitative dose-response and time-course information. A relevant new development in this respect are so-called quantitative AOP (qAOP) approaches that focus on the simulation of the dynamic processes linking a MIE with an adverse outcome using different modelling approaches (Conolly et al., 2017; Schultz and Watanabe, 2018; Zgheib et al., 2019). Such qAOPs can be linked to PB(P)K modelling results. The *in vitro* exposure that is expected to perturb a MIE or KE would then provide a starting point to estimate an external exposure scenario of possible concern. Therefore, the development of qAOPs will be an important next step to link biokinetics and toxicodynamics within next-generation risk assessment (Edwards et al., 2015; Vinken, 2018).

6 Conclusion

Figure 1 provides a schematic overview of the workshop results. Overall, it can be concluded that opportunities for the implemen-

tation of NAMs for biokinetics in current regulatory risk assessment are to

- evaluate interspecies differences,
- simulate special populations and human interindividual variability,
- perform read-across,
- perform route-to-route extrapolations,
- set priorities (e.g., exposure-based waiving),
- perform dose extrapolations, and
- guide *in vivo* toxicity test selection and design.

When switching to a fully animal-free regulatory risk assessment for humans in the future, these methods will be necessary to also

- guide *in vitro* toxicity test selection and design, and
- enable QIVIVE.

Given the importance of NAMs for biokinetics in a successful transition towards next-generation toxicity testing strategies, both regulatory and scientific experience with *in vitro/in silico* biokinetic approaches must increase. To facilitate the use of human-relevant NAMs for biokinetics, GDs are needed that provide information on

- how NAMs for biokinetics should be designed to address a regulatory topic, including, for example, how the different parameters should be scaled and integrated in a kinetic model,
- the essential kinetic parameters that are needed to address the regulatory topic, and
- the applicability domain of the different methods available for each of these parameters,
- the use of different existing *in silico* tools to calculate, e.g., partition coefficient, fraction unbound plasma or blood:plasma ratios, and
- how to link NAMs for biokinetics and for toxicodynamics for risk assessment purposes.

In addition, the validation of NAMs for biokinetics and the development of OECD TGs (on how to perform a specific biokinetic study) is important. At the workshop, the development of OECD TGs was prioritized based on the availability of current methods and research needs. It was recognized that OECD TGs are required for:

- intrinsic clearance and identification of metabolites (liver and, at later stages, also other organs),
- *in vitro* approaches for tissue binding (e.g., fraction unbound plasma, blood:plasma ratio), and
- (passive) intestinal absorption (at later stages also passive and active permeation into other organs). In case of *in vitro* skin absorption, a TG already exists, which may need to be developed further.

Before TGs can be developed for each of these kinetic processes, protocols are needed that lead to robust and reproducible results. Moreover, it is advised to have a continuous OECD expert group to perform this work on GDs and TGs. In the meantime, the OECD Expert Group on Toxicokinetics (previously Ad hoc OECD Expert Group on Biotransformation) has been established.

Furthermore, it was concluded that method development is required to

- adequately address transporter mediated absorption (intestine, lung), distribution (e.g., blood-brain barrier, placenta, organ



uptake) or excretion (e.g., active renal excretion, biliary secretion),

- obtain cell models that reflect the physiology and kinetic characteristics of different organs, e.g., expression of transporters and metabolizing enzymes.

Recent progress in the fields of immortalized primary cells, iPSCs, ASCs or ESCs, 3D organoids and body-on-a-chip systems provide promising tools to meet these research needs. To facilitate the transition towards next-generation risk evaluations, NAMs within the field of biokinetics should be linked to conceptual frameworks, such as (quantitative) AOPs.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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