Regulatory Toxicology and Pharmacology 99 (2018) 5–21

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

Finding synergies for 3Rs – Toxicokinetics and read-across: Report from an EPAA partners' Forum

Charles Laroche[a], Manoj Aggarwal[b], Hans Bender[c], Paul Benndorf[d], Barbara Birk[e], Jonathan Crozier[f], Gianni Dal Negro[g], Federica De Gaetano[h], Christian Desaintes[i], Iain Gardner[j], Bruno Hubesch[k], Amaia Irizar[a,*], David John[l], Vikas Kumar[m], Alfonso Lostia[n], Irene Manou[f], Mario Monshouwer[o], Boris P. Müller[p], Alicia Paini[n], Kirsty Reid[q], Timothy Rowan[r], Magdalini Sachana[s], Katrin Schutte[t], Catrina Stirling[u], Rob Taalman[v], Leon van Aerts[w], Renate Weissenhorn[h], Ursula G. Sauer[x]

[a] *The International Fragrance Association (IFRA), Belgium*
[b] *Dow AgroSciences, United Kingdom*
[c] *Bonn, Germany*
[d] *Henkel AG & Co KGaA, Germany*
[e] *BASF SE, Germany*
[f] *European Partnership for Alternative Approaches to Animal Testing (EPAA) Industry Secretariat, Belgium*
[g] *GlaxoSmithKline, United Kingdom*
[h] *European Commission, the Directorate-General (DG) for Internal Market, Industry, Entrepreneurship and SMEs (GROW), Belgium*
[i] *European Commission, The Directorate-General (DG) for Research and Innovation (RTD), Belgium*
[j] *SIMCYP, United Kingdom*
[k] *Hubesch Consult BVBA, Belgium*
[l] *Animal Health Europe, Belgium*
[m] *University of Rovira i Virgili, Spain*
[n] *European Commission, The Directorate-General (DG) Joint Research Centre (JRC), Italy*
[o] *Janssen Pharmaceuticals, Belgium*
[p] *Symrise AG Holzminden, Germany*
[q] *European Federation of Pharmaceutical Industries and Associations (EFPIA), Belgium*
[r] *EPAA Consultant, United Kingdom*
[s] *Organisation for Economic Cooperation and Development (OECD), France*
[t] *European Commission, the Directorate-General (DG) Environment (ENV), Belgium*
[u] *Zoetis Ltd, United Kingdom*
[v] *Cosmetics Europe, Belgium*
[w] *Medicines Evaluation Board, the Netherlands*
[x] *Scientific Consultancy – Animal Welfare, Germany*

Abbreviations: ADME, Absorption, distribution, metabolism, elimination / excretion; AMBIT, [Open chemo-informatic system designed to facilitate chemical safety assessment]; AOP, Adverse outcome pathway; BE, Biomonitoring equivalent; BP, Biocidal product; CEFIC, European Chemical Industry Council; CHMP, Committee for Human Medicinal Products; CLP, Classification; Labelling and Packaging, CSAF; Chemical specific adjustment factor, DG; General Directorate, EC; European Commission, ECHA; European Chemicals Agency, EFPIA; European Federation of Pharmaceutical Industries and Associations, EFSA; European Food Safety Authority, EMA; European Medicines Agency, ENV; Environment (DG), EP; European Parliament, EPAA; European Partnership for Alternative Approaches to Animal Testing, EURL ECVAM; European Union Reference Laboratory for Alternatives to Animal Testing, GROW; Internal Market, Industry, Entrepreneurship and SMEs (DG), IATA; Integrated approach for testing and assessment, ICH; International Conference on Harmonisation, IFRA; International Fragrance Association, IMI; Innovative Medicines Initiative, iTTC; Internal threshold of toxicological concern, IUCLID; International Uniform Chemical Information Database, IVIVE; *In vitro*, *in vivo* extrapolation; JRC, Joint Research Centre (DG); LRI, Long-Range Research Initiative; LRSS, Long Range Science Strategy; MEGen, Physiologically based pharmacokinetic model generator; MoA, Mode-of-action; NOAEL, No-observed-adverse-effect level; OECD, Organisation for Economic Co-operation and Development; PB(P)K, Physiologically based (pharmaco-) kinetic; PPP, Plant protection product; QSAR, Quantitative Structure Activity Relationship; R&D, Research and development; REACH, Registration, Evaluation, Authorisation and Restriction of Chemicals; RfC, Reference concentration RIVM; National Institute for Public Health and the Environment, The Netherlands; RTD, Research and Innovation (DG); RVis: R Visual [open-source modelling platform for a biologically based, quantitative risk assessment of chemicals]; SCCS, Scientific Committee on Consumer Safety; SEURAT-1, Safety Evaluation Ultimately Replacing Animal Testing; SME, Small and medium-sized enterprises; TG, Test guideline; TIMES, Tissue metabolism simulator; TTC, Threshold of toxicological concern; UF, Uncertainty factor; WoE, Weight-of-evidence

* Corresponding author. The International Fragrance Association (IFRA), Ave des Arts 6, 1210, Brussels, Belgium.
E-mail address: airizar@ifraorg.org (A. Irizar).

https://doi.org/10.1016/j.yrtph.2018.08.006
Received 25 May 2018; Received in revised form 17 July 2018; Accepted 16 August 2018
Available online 23 August 2018

ARTICLE INFO

Keywords:
Physiologically-based kinetic (PBK) modelling
Hazard and risk assessment
Weight-of-evidence (WoE)
Threshold of toxicological concern (TTC)
In vitro to *in vivo* extrapolation (IVIVE)
Integrated approach for testing and assessment (IATA)
Absorption
Distribution
Metabolism
And elimination
Excretion (ADME)

ABSTRACT

The European Partnership for Alternative Approaches to Animal Testing (EPAA) convened a Partners' Forum *Toxicokinetics and Read-Across* to provide an overview on research activities to develop *in vitro* toxicokinetics methods and physiologically-based kinetic (PBK) models and to find synergies to enhance use of toxicokinetic data to strengthen read-across. Currently, lacking toxicokinetic data often prevent the application of read-across. Preferably, toxicokinetic data should be generated using *in vitro* and *in silico* tools and anchored towards human relevance. In certain sectors, PBK modelling is being used for risk assessment, but less so in others. Specific activities were identified to facilitate the use of *in vitro* and *in silico* toxicokinetic data to support read-across: The collation of available tools indicating the parameters and applicability domains covered; endpoint-specific guidance on toxicokinetics parameters required for read-across; case studies exemplifying how toxicokinetic data help support read-across. Activities to enhance the scientific robustness of read-across include the further user-friendly combination of read-across tools and formal guidance by the authorities specifying the minimum information requirements to justify read-across for a given toxicity endpoint. The EPAA was invited to continue dissemination activities and to explore possibilities to collate a contemporaneous list of open toxicokinetics tools that assist risk assessment.

1. Introduction

Read-across implies predicting toxicological endpoint information for one substance, the target substance, by using existing data for the same endpoint from one or more other substances, the source substance (s) (ECHA, 2017a); *cf.* Section 5 **- Glossary** for definitions and explanatory notes to key terms used in this report. Read-across approaches are increasingly being taken into consideration for substance hazard and risk assessment as a means to effectively exploit all available data (Patlewicz et al., 2015). Read-across is expected to contribute considerably to the 3Rs principle to replace, reduce, and refine animal testing (Russell and Burch, 1959) that has been implemented in *Directive (2010)/63/EU on the protection of animals used for scientific purposes* (EP and Council, 2010). Further, the successful use of read-across approaches is expected to help speed up the research and development (R &D) processes and regulatory decisions (Ball et al., 2016; ECHA, 2016).

To date, a barrier to the successful use of read-across for regulatory purposes is establishing that the toxicokinetic behaviour of the source and target substance(s) are sufficiently similar to substantiate the validity of the read-across (Punt et al., 2011; Ball et al., 2016). A key question to address is which differences in chemical structure between the source and target substances affect the toxicokinetics to a degree that would invalidate the read-across justification (Hand et al., 2017; Schultz and Cronin, 2017). Further, although *in silico* and *in vitro* methodologies for predicting toxicokinetic properties are available, these are not necessarily sufficiently advanced to replace *in vivo* studies for regulatory hazard and risk assessment. (In the present report, the term 'risk assessment' is used for consistency purposes throughout, even though in some sectors (and regulations) the term 'safety assessment' is more commonly employed instead.)

To enhance coordination among the different industry sectors and to facilitate the successful use of read-across for regulatory purposes, the European Partnership for Alternative Approaches to Animal Testing (EPAA) organised the EPAA Partners' Forum *Finding synergies for* 3Rs - *Toxicokinetics and read-across* that took place on 21 November 2017 in Brussels, Belgium. The EPAA is a voluntary collaboration between the European Commission (EC) and companies and European trade associations from eight industry sectors. The EPAA partners share the vision to enhance application of the 3Rs principles for meeting regulatory hazard and risk assessment requirements while maintaining the balance between the safety of products, animal welfare and scientific innovation.

The EPAA Partners' Forum *Finding synergies for* 3Rs - *Toxicokinetics and read-across* was conceived as a cross-sector research coordination instrument providing the members of the EPAA the opportunity to exchange information about their respective activities in relevant sectorial flagship initiatives. Examples for such flagship initiatives are the European Chemical Industry Council (CEFIC) Long-Range Research Initiative (LRI; de Boer et al., 2015); the Cosmetics Europe Long Range Science Strategy (LRSS) on Alternative Approaches to Animal Testing; and, in the pharmaceutical sector, the Innovative Medicines Initiative (IMI; Goldman et al., 2015), an EU partnership for health-related research with public and private EU funding under the 7th Research Framework Programme and the Horizon 2020 Research Framework Programme.

It was the aim of the EPAA Partners' Forum to provide a comprehensive picture of relevant ongoing activities and to progress the development of predictive toxicokinetics methods aligned with the 3Rs principle and the use of data from such methods within read-across approaches. Discussions revolved around sessions covering the spectrum of stakeholders working on toxicokinetics and read-across: regulators, the regulated from all relevant industry sectors, method developers and downstream users. The proceedings of the EPAA Partners' Forum are presented in this report that serves (i) to provide a comprehensive overview of the discussions; (ii) to build a consensus on the role toxicokinetic data play and should ideally play in read-across; and (iii) to showcase the actions defined during the Forum as the way forward to find synergies for 3Rs on toxicokinetics and read-across.

Thirty-one invited participants attended the EPAA Partners' Forum representing the EC Directorates-General (DGs) Environment (ENV); Internal Market, Industry, Entrepreneurship and SMEs (GROW); Joint Research Centre (JRC); and Research and Innovation (RTD); the European Food Safety Authority (EFSA); the Medicines Evaluation Board of the Netherlands (representative of the European Medicines Agency (EMA)); the Organisation for Economic Cooperation and Development (OECD); as well as companies from the chemicals, pharmaceuticals and vaccines, cosmetics, soaps and detergents, crop protection, animal health, and fragrances sectors and their European trade federations (*cf.* Supplementary Information for the list of participants). *Hans Bender (Germany)* chaired the EPAA Partners' Forum and moderated the discussions.

2. Presentations from the EPAA partners' Forum

2.1. Setting the scene

The current EPAA Industry Co-Chair, *Charles Laroche (International Fragrance Association (IFRA), Belgium)* highlighted the importance of the EPAA Partners' Forum in collating advances made in all relevant fields of science related to the use of read-across approaches and to the

in vitro and *in silico* assessment of toxicokinetics. Due to the unique range of partners in the EPAA, scientific knowledge gaps preventing the use of data from *in vitro* and *in silico* toxicokinetics tools within read-across approaches would be revealed, and synergies between sectors and funding opportunities to address such knowledge gaps identified. Further, mechanisms for the future regular exchange of information would be established to optimise the use of resources in advancing *in vitro* and *in silico* toxicokinetics tools and their application in read-across approaches.

2.2. Toxicokinetics strategy of the JRC and ongoing activities of the OECD

2.2.1. Toxicokinetics strategy of the JRC

Alfonso Lostia (EC DG JRC, Italy) outlined the legal framework for collating toxicokinetic data implemented in the EU. Whereas information on toxicokinetics plays an important role in hazard and risk assessment, such information is only mandatory under the Plant Protection Products (PPP) and Biocidal Products (BP) Regulations (*Regulation (EC) No 1107/2009 on the placing of PPPs on the market* (EP and Council, 2009a) and *Regulation (EU) No 528/2012 concerning the making available on the market and use of BPs* (EP and Council, 2012)). In these Regulations, the fulfilment of standard information requirements mainly relies on *in vivo* data, even though both include general provisions to minimise animal testing (Articles 8 and 18 of the PPP Regulation and Article 62 of the BP Regulation). Further, for PPP, a new data requirement has been included in 2015 to perform *in vitro* comparative metabolism studies using human and animal material to assess potential species differences in toxicological data (Commission, 2013).

For cosmetics, information on toxicokinetics parameters (human systemic and dermal exposure, and biotransformation) is 'recommended' (*Regulation (EC) No 1223/2009 on cosmetic products* (EP and Council, 2009b)), whereas it is not a standard information requirement for chemicals (*Regulation (EC) No 1907/2006 concerning the registration, evaluation, authorisation and restriction of Chemicals* (REACH; EP and Council, 2006) or for substance classification, labelling and packaging (CLP; *Regulation (EC) No 1272/2008 on the CLP of substances and mixtures* (EP and Council, 2008)). Based on the 3Rs principle, specific use scenarios for ADME information are suggested in different EU guidance documents allowing, e.g., to waive a specific *in vivo* study in the absence of systemic absorption. Finally, the European Chemicals Agency (ECHA) report on alternatives (ECHA, 2017b) mentions toxicokinetics models as basic elements of integrated approaches to testing and assessment (IATAs).

The *European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) Strategy for achieving* 3Rs *impact in the assessment of toxicokinetics and systemic toxicity* (Bessems et al., 2015) describes opportunities to generate toxicokinetic data in human hazard and risk assessment while ultimately avoiding the need for animal studies. This toxicokinetics strategy has identified four main aims to facilitate the generation, acceptance and use of *in vitro* and *in silico* toxicokinetic data for regulatory purposes.

Aim 1 strives to advance *in vitro* methods that can provide information that is relevant for assessing either absorption, distribution, metabolism or elimination. Since metabolism is frequently the main driver of toxicokinetics, ongoing research undertaken at the JRC focuses on *in vitro* methods that can provide information on hepatic metabolic clearance. This work also contributes to the development of an *OECD Guidance Document (GD) for characterisation and description of in vitro human hepatic metabolic clearance methods*. This OECD GD will provide a framework focused on identifying elements that are relevant for characterising and describing *in vitro* hepatic metabolic clearance methods, in order to support the assessment of their performance and to facilitate their inter-comparison. Thereby, the OECD GD is expected to enhance the communication between *in vitro* method developers, users and regulators to increase confidence in the use of these methods to support substance hazard and risk assessment.

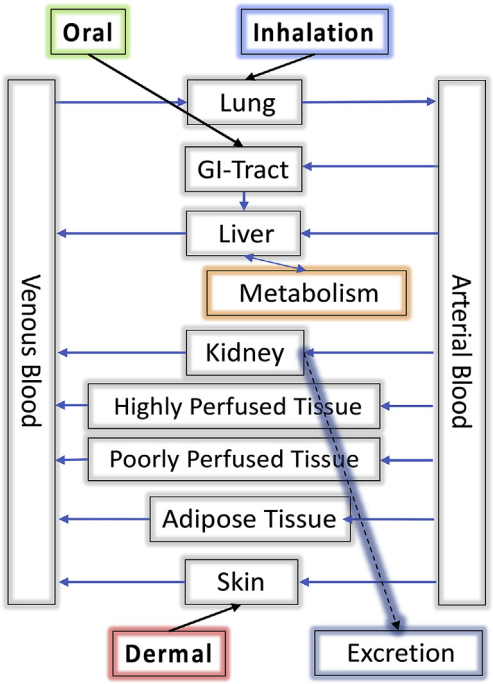

Fig. 1. Schematic of a PBK model that uses a compartmentalised structure where different compartments represent target organs that are interconnected and described mathematically.
Portals of entry via the oral, inhalation and dermal route are indicated with black continuous arrows; excretion (via the kidneys) is indicated with an interrupted arrow. Arrows in blue reflect biodistribution within the organism.

Aim 2 encompasses activities to establish good practices in physiologically based kinetic (PBK) modelling. (In this report, PBK is used as standard term for modelling to describe the fate of a substance in the organism by mathematical equations. Further, the term physiologically-based pharmacokinetic (PBPK) is used if such modelling specifically relates to pharmacokinetics; *cf.* also the respective explanatory notes in Section 5 **– Glossary.**) As a new generation of PBK models will be developed in the absence of *in vivo* data, relying solely on *in vitro* and *in silico* data, it is necessary to identify current challenges in constructing, validating and applying these models. This new generation of PBK models (Fig. 1) reflects a mechanistic understanding of biochemical processes and biological pathways. In pursuing Aim 2, an *EURL ECVAM Workshop on new generation of PBK models in risk assessment* was convened in 2016 to establish a workflow for Good Kinetic Modelling Practices. Sources of uncertainty in PBK models were identified, and a long-term strategy to facilitate the use of these models for risk assessment was established (Paini et al., 2017a).

For this purpose, the EURL ECVAM, together with the U.S. Environmental Protection Agency (EPA), the Oak Ridge Institute (USA) and the consultancy Klimeto (Slovakia), conducted a survey on the application of PBK models in science and regulatory submissions (Paini et al., 2017b). The respondents identified the following major obstacles preventing the regulatory use of data from PBK modelling: (i) Unavailability of data for model validation; (ii) lack of user-friendly software and guidance; (iii) lack of expertise within the regulatory agencies; and (iv) differences in acceptance criteria between agencies and countries.

The outcome of the EURL ECVAM workshop and the survey will contribute to the development of an *OECD GD on the characterisation, validation and reporting of physiologically based models for regulatory applications* that is currently on the OECD work programme, co-led by the EU (JRC) and the USA (EPA).

Aim 3 serves the collection of toxicokinetic data (*cf.* Table 1 for an unexhaustive list of potential sources for toxicokinetic data).

Table 1
Sources of toxicokinetic data.

Type of data	Source	Link or reference
Human *in vitro* ADME data	JRC EURL ECVAM Database Service on Alternative Methods to Animal Experimentation	https://ecvam-dbalm.jrc.ec.europa.eu/
	JRC Quantitative Structure Activity Relationship (QSAR) database	https://eurl-ecvam.jrc.ec.europa.eu/databases/jrc-qsar-model-database
Human *in vivo* toxicokinetic data	ECVAM KinPar database	https://eurl-ecvam.jrc.ec.europa.eu/validation-regulatory-acceptance/docs-toxicokinetics/ECVAM%20KinParDB%20-%20Short%20manual%20-2.pdf
	Online document from the National Institute for Public Health and the Environment (RIVM), NL: Data collection on kinetic parameters of substances	https://eurl-ecvam.jrc.ec.europa.eu/validation-regulatory-acceptance/docs-toxicokinetics/Methodological%20report.pdf (Noorlander et al., 2008)
Mammalian *in vivo* data	eChem Portal	https://www.echemportal.org/echemportal/index.action
	QSAR Toolbox	https://www.qsartoolbox.org/
	MetaPath	http://oasis-lmc.org/products/software/metapath.aspx
Anatomical and physiological data	RIVM interspecies database	https://www.interspeciesinfo.com/
Knowledgebase	PBPK Knowledgebase	(Lu et al., 2016)
	Adverse Outcome Pathways-Knowledgebase • eAOP Portal • AOP wiki	• https://aopkb.oecd.org/ • https://aopwiki.org/
	Effectopedia	https://www.effectopedia.org/
In vitro methods and data	TOX Bank, output from the research initiative SEURAT-1	http://toxbank.net/
	ToxCast high-throughput toxicology	https://www.epa.gov/chemical-research/toxicity-forecasting
	Toxicology in the 21st Century	https://www.epa.gov/chemical-research/toxicology-testing-21stcentury-tox21
	R package for high-throughput toxicokinetics	(Pearce et al., 2017)
Biomonitoring data	IPCheM - the Information Platform for Chemical Monitoring (hosted by JRC)	https://ec.europa.eu/jrc/en/event/conference/ipchem
	EXPOCAST (U.S. Environmental Protection Agency)	https://cfpub.epa.gov/si/si_public_record_Report.cfm?dirEntryId=211811

Aim 4 serves to facilitate the regulatory anchoring of *in vivo* human ADME with *in vitro* data (from human and/or animal cell lines) and *in silico* toxicokinetic data that are integrated within an IATA. Case studies are needed to show how such IATAs can be put into practice. However, the integration of different *in vitro* and *in silico* tools is challenging, and decision-making frameworks for such integration are still under discussion.

Overall, the successful implementation of the EURL ECVAM toxicokinetics strategy will require the collective and coordinated contribution of a wide range of international stakeholders.

2.2.2. Ongoing activities of the OECD

Magdalini Sachana (OECD, France) explained that, for many years, the OECD has been developing tools and guidance for the use of alternative methods, such as Quantitative Structure Activity Relationships (QSARs), chemical categories and IATAs. In 2014, the *OECD Joint Meeting of the Chemicals Committee and Working Party on Chemicals, Pesticides, and Biotechnology* initiated the IATA case studies project to enhance the applicability of these methodologies (*cf.* http://www.oecd.org/chemicalsafety/risk-assessment/iata-integrated-approaches-to-testing-and-assessment.htm). Specifically, this project aims at developing IATAs that are globally accepted for regulatory decision-making, and at preparing an OECD-agreed framework for developing and using IATAs, building on alternative methods and adverse outcome pathways (AOPs). The IATA case study project has completed three review cycles: During the first and second review cycles, case studies on how to include grouping methods within IATAs were collated and evaluated (OECD, 2016a, 2017a). Uncertainties in the outcome of the assessment when applying grouping methods are often caused by a lack of toxicokinetic parameters so that, e.g., the level of similarity in metabolism between substances cannot be established. The review cycles further emphasised the need to develop guidance for how to assess the reliability of data from *in vitro* methods and from PBK models that were solely built with *in vitro* data and *in silico* predictions. Similarly, guidance should be developed on the estimation of internal doses, *in vitro* to *in vivo* extrapolations (IVIVE), metabolism, and clearance. Such guidance should illustrate how the credibility of models can be established in the context of different regulatory applications.

Additional OECD GDs that are relevant for read-across and toxicokinetics include OECD GD 194 (OECD, 2014), which provides general guidance on the grouping of chemicals. Its Section 6.2 is specifically dedicated to the topic of metabolic or degradation pathways and toxicokinetics. OECD GD 229 (OECD, 2015) presents fundamental and guiding principles for QSAR analysis of chemical carcinogenicity with mechanistic considerations. Therein Section 2.1 addresses toxicokinetics and toxicodynamics as the key components of mechanism-based QSAR analyses.

Finally, within the ongoing fish hepatic metabolism project, two OECD Test Guidelines (TGs) on the calculation of *in vitro* intrinsic clearance via rainbow trout S9 mix or cryopreserved hepatocytes, respectively, are expected to be finalised by the end of 2018. In the area of skin absorption testing, OECD GD 28 for the conduct of skin absorption studies (OECD, 2004a) and the OECD guidance notes on dermal absorption (OECD, 2011) are currently being updated, and an update of OECD TG 428 on *in vitro* skin absorption (OECD, 2004b) is under consideration.

2.3. Ongoing sectorial activities

2.3.1. Pharmaceuticals and vaccines sector input

Representing the European Federation of Pharmaceutical Industries and Associations (EFPIA), *Mario Monshouwer (Janssen Pharmaceuticals, Belgium)* exemplified challenges faced by the pharmaceutical industry when performing toxicokinetics and pharmacokinetics assessments. Pharmacokinetics are of unique relevance for the pharmaceutical industry and serve to determine the efficacious, safe and convenient dose range of a compound during drug discovery and development. By comparison, it is the primary objective of toxicokinetic studies to describe the systemic exposure achieved in animals and its relationship to dose levels and the time course of the toxicity study. Therefore,

toxicokinetics in combination with preclinical data are critical for defining margins-of-safety and impact the starting and ceiling doses (exposures), for first-in-human trials.

Using *in vitro* predictions of human exposure and animal toxicokinetics to support a decision regarding margins-of-safety remains challenging. In addition, these challenges may differ based on the therapeutic modality (i.e. small molecules, recombinant proteins, peptides, monoclonal antibodies). Nevertheless, over recent years, significant progress has been made in using PBPK modelling to predict human pharmacokinetics. In particular, in the area of drug-drug interaction there are now numerous examples of PBPK-based simulations that have been accepted by health authorities and are included in drug labels (Shebley et al., 2018). Animal data continue to be an important aspect of PBPK model building and verification. In particular, in the absence of any clinical data, it is crucial to establish *in vitro - in vivo* correlations based on animal data to build confidence in human pharmacokinetics and exposure predictions.

Besides the determination of toxicokinetic and pharmacokinetic parameters (based on accurate exposure-time profile) of the parent drug, it is also essential to obtain accurate plasma exposure profiles of metabolites. Whenever a metabolite is present to a considerable extent in humans, it has to be characterised non-clinically, adhering to the International Conference on Harmonisation (ICH) *Guidance M3 (R2) on Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorisation for Pharmaceuticals* (EMA, 2009). This often implies additional animal studies, as discussed in an American Association of Pharmaceutical Scientists meeting report *Metabolites in Safety Testing* (Gao et al., 2013).

Although significant progress has been made in PBPK-based human/animal exposure predictions using conventional pharmaceutical formulations, it remains challenging to obtain accurate predictions when very specific pharmaceutical formulations are used to maximise exposure to the compound. Also, it is important to note that very small chemical changes can have a considerable impact on exposure profiles. For instance, there are large differences in the clearance of deuterated versus non-deuterated carbazeran (Sharma et al., 2012) or of (R)- versus (S)-etodolac (de Miranda Silva et al., 2017). Therefore, great care has to be taken in using structural similarities to guide exposure predictions.

Further, it is important to understand the potential for saturation of ADME processes and to correctly identify and take into account substance- and/or disease-induced events that could impact ADME. This might be the case when, e.g., the substance and/or disease affects the intestinal transit time, liver blood flow, or liver physiology. Obviously, such scenarios make *in silico*-based predictions challenging. Nevertheless, for many types of substances, the predictions derived from PBPK modelling are satisfactory. As Dr. Monshouwer explained, the current aim is to obtain a less than three to four-fold difference between predicted and observed human exposure data. Beyond the establishment of PBPK models, changes in toxicity study designs have had a substantial impact on reducing animal testing. By reducing the frequency of blood sampling and the sample volumes for toxicokinetic assessments, the need to include satellite dosing groups in toxicity tests is becoming obsolete (Chapman et al., 2013; ICH, 2017).

2.3.2. Cosmetics sector input

Rob Taalman (Cosmetics Europe, Belgium) presented the Safety Assessment Framework developed within the research initiative *Safety Evaluation Ultimately Replacing Animal Testing* (SEURAT-1; http://www.seurat-1.eu/; Gocht et al., 2015; Berggren et al., 2017). This framework serves to ensure best possible use of *in vitro* and *in silico* data. During Tier 0, all available data are collected and product use scenarios identified. The available data are evaluated to determine applicable thresholds of toxicological concern (TTC). If exposure to the substance under investigation is below the TTC value, the risk can be considered sufficiently low and further toxicological testing is not deemed necessary. Further, the available data are evaluated to identify analogues and opportunities to apply read-across to substantiate the conclusion (Berggren et al., 2015).

If risk cannot be excluded in Tier 0, Tier 1 serves to formulate a hypothesis for the further weight-of-evidence (WoE) evaluation. The hypothesis addresses the potential systemic availability of the parent compound and/or its metabolites, as well as potential target organs and the internal concentration at such target sites, taking into account mode-of-action (MoA) considerations, as applicable. Internal TTC (iTTC) values can be generated to assess internal concentrations. For this purpose, 'internal' NOAELs are derived by multiplying external NOAELs with predicted (or measured) bioavailability estimates for the given substance (Partosch et al., 2015). If the internal concentration is low, further testing might not be necessary. In Tier 2, the need for targeted testing is determined on a case-by-case basis. With respect to toxicokinetics, testing is likely to begin with dermal penetration studies and the evaluation of substance clearance. Tier 2 testing can be complex if the substance is metabolised and considerable amounts of one or more specific metabolites evolve.

In the cosmetics sector, ongoing research projects related to toxicokinetics focus on skin penetration and, secondarily, the kinetics in the respiratory tract as a further potential portal of entry (Gerstel et al., 2016; Jaques-Jamin et al., 2017; Rothe et al., 2017a). In an ongoing research project assessing skin bioavailability conducted in the context of the Cosmetics Europe LRSS, all available data for this endpoint have been collected and used to assess available *in silico* models. Two *in silico* skin penetration models were selected as sufficiently advanced to merit further development. The use of *in silico* models will contribute to the evaluation of skin absorption at higher throughput and with less resources than when performing *ex vivo* skin penetration studies (Rothe et al., 2017b).

Consideration of the iTTC, supported by systemic exposure predictions, is increasingly gaining importance for the assessment of cosmetic substances. There are scenarios where the internal dose is more relevant than the external dose, e.g. during read-across approaches assessing the toxicity of a metabolite of a non-toxic parent compound. Consideration of the internal dose is also relevant to justify exposure-based waiving of testing and to assess low-level substance exposure from more than one exposure route.

Different ongoing Cosmetics Europe projects support the iTTC concept and its use for risk assessment. These projects aim at using available PBK models that are often first developed for the assessment of orally applied pharmaceuticals so that they have to be expanded to cover dermal exposure (Gajewska et al., 2015; Klein et al., 2016; Bessems et al., 2017). Specifically, an *in vitro* ADME Toolbox is being developed to provide input data for a PBK platform that includes topical exposure. As with all extrapolations from animal-derived data to humans, it remains difficult to ensure that the PBK-based predictions are relevant. For instance, the clearance rates for a given substance might differ considerably between rats and humans (Wood et al., 2017; Horiuchi et al., 2018). An understanding of such species-specific differences is important to improve PBK modelling.

Within a biokinetic project, ongoing activities aim at designing *in vitro* methods that allow measuring where the applied substance is located within the test system and which proportion of the applied dose comes into contact with the cultured cells within the given incubation time. Such *in vitro* dosimetry information is pivotal for the interpretation of *in vitro* effects, i.e. to correlate *in vitro* concentrations to *in vivo* concentrations, and to undertake IVIVE (Wambaugh et al., 2018).

Finally, a multi-organ-chip (skin and liver) model project undertaken by Cosmetics Europe aims at developing static and dynamic 3D skin and liver models to measure the effect of single versus repeated application on substance metabolism and route-to-route differences. While the testing of cosmetic ingredients is focused on dermal effects and dermal penetration, an understanding of effects on the liver is relevant for substances that become systemically available.

Together, these projects aim at providing a combination of *in silico* and *in vitro* data that will help understand the bioavailability and metabolism of topically applied compounds. The integration of PBK modelling with the iTTC concept is expected to promote the latter as a tool for risk assessment. It is planned to integrate toxicokinetic data with toxicodynamics data to help inform different case study scenarios (TTC, read-across, *ab initio* testing). These case studies are expected to contribute to a tripartite dialogue between test method developers, users, and regulators on the applicability of *in silico* modelling, *in vitro* assays, TTC and read-across. To warrant the meaningfulness of the case studies, the specific biological questions under investigation must be clearly defined in the context of the Safety Assessment Framework, and the case study substances selected accordingly. In the cosmetics sector, this latter task can be challenging when cosmetic ingredients are of very low toxicity, so that toxicological effects only evolve at extremely high concentrations, and MoAs cannot be identified.

2.3.3. Fragrances sector input

Amaia Irizar (IFRA, Belgium) outlined that, within the Fragrance Safety Assessment Programme, risk management is communicated in the form of IFRA Standards that are based on a systematic review of the properties of fragrance materials carried out by the U.S.-based Research Institute of Fragrance Materials (RIFM; Api et al. (2015); *cf.* http://www.ifraorg.org/en-us/standards#.Whu487aZPOQ). In the Fragrance Safety Assessment Programme, non-animal methods are implemented as far as possible. The assessment of a fragrance begins by collecting available data and determining their adequacy for risk assessment (Step 1). If those data are considered insufficient, in Step 2, read-across approaches are applied and *in vitro* testing and *in silico* modelling is performed for each toxicological endpoint, using tools that are specific for the given endpoint. Should these additional considerations not allow ensuring the safe use of the substance *per se*, in Step 3, a comparison of its exposure to a TTC is determined. As a last resort, in Step 4, additional data are generated, which could include data on the substance's metabolic pathway or on analogues that are relevant for read-across. Such analogues are identified following the strategy for structuring and reporting a read-across prediction of toxicity described in Schultz et al. (2015) that stands in line with the ECHA Read-Across Assessment Framework (ECHA, 2017a) and with relevant OECD GDs (OECD, 2014, 2016a).

IFRA members use read-across both during R&D and for regulatory submissions. In the experience of the industry members, toxicokinetics based on theoretical reasoning, with significant reliance on *in silico* toxicokinetic data, is key to show similar skin absorption, bioavailability, and metabolism to support read-across. Generally, bioavailability is predicted taking into account the substance's molecular weight and physico-chemical properties. Skin absorption is evaluated using the Skin Absorption Model described by Shen et al. (2014) that allows calculating the maximum flux by integrating data from QSAR models that determine octanol/water partition coefficient, water solubility and permeability coefficient. This model was successfully validated with a large fragrance-focused data set containing 131 materials (Shen et al., 2014). Metabolism is predicted using a variety of different tools (Box).

In vitro models are used, e.g., to investigate substance metabolism in hepatocytes. However, at present such *in vitro* data are generally not used for risk assessment and read-across due to prevailing difficulties in translating *in vitro* doses to *in vivo* doses. Similarly, although data from *in vitro* skin absorption studies (OECD TG 428; OECD, 2004b) are used for risk assessment, they are generally not used for read-across.

A research project planned by RIFM is related to the topic of *in vitro* and *in silico* toxicokinetics of fragrance materials. This project aims at assessing the Caco-2 permeability assay (Press, 2011) and GastroPlus™ PBPK modelling (available at: http://www.simulations-plus.com/software/gastroplus/) to determine internal exposures to fragrance materials. However, at present this work does not focus on application for read-across.

Within the fragrances sector, *in vitro* and *in silico* toxicokinetic tools are mostly used during R&D for internal evaluations. Their regulatory use is encumbered by the fact that many of the tools have not yet gained regulatory acceptance. Uncertainties in applying *in vitro* and *in silico* toxicokinetics tools are caused, e.g., by difficulties in extrapolating *in vitro* metabolism data to a physiological response and in identifying false positives in predicted metabolites. The regulatory applicability of *in vitro* and *in silico* toxicokinetics tools could be facilitated by the provision of an expanded training set for QSAR metabolism prediction models and by advancing QSAR tools to include simulations of metabolism rates. Finally, guidance is required on how to use PBK-based predictions within read-across approaches.

2.3.4. Agrochemicals sector input

Manoj Aggarwal (Dow AgroSciences, United Kingdom), speaking on behalf of the European Crop Protection Association, explored the regulatory drivers to collect toxicokinetic data in the agrochemicals sector. Toxicokinetic data play a key role in the WoE evaluation to select appropriate dose levels for toxicity studies, i.e. the kinetically-derived maximum dose. Since toxicokinetics studies are generally conducted at very high and potentially toxic doses, the dose-response relationship of one or more ADME processes may be saturated (indicated by nonlinear dose-response relationships). To avoid animal suffering and to ensure relevance of the data for human health risk assessments, the appropriate dose levels for toxicity studies are selected in linear kinetic concentration ranges.

Research activities within the agrochemicals sector have aimed at adapting the study design of regulatory toxicity tests to allow integrating the sampling of tissues and body fluids for toxicokinetics assessments into these tests (Saghir et al., 2012). Dow AgroSciences has developed a toxicokinetics framework that takes into account the substance's internal dose during all steps of risk assessment, i.e. (i) hazard identification; (ii) dose-response assessment; (iii) exposure assessment; (iv) risk characterisation; and (v) risk management (Fig. 2). By comparison, traditional risk assessment only takes into account the substance's external dose. Consideration of the internal dose (i.e., toxicokinetics) ensures that the available information on the effects that the substance can elicit in the organism is fully exploited, and it is also a prerequisite for substance biomonitoring. In the early steps of the toxicokinetics framework, a comparative metabolism analysis allows determination of species differences in metabolism (Terry et al., 2016; Whalley et al., 2017). Toxicokinetic data could serve to bridge route-to-route extrapolations and *in vitro* (or *in silico)* toxicity data to animal data. Based upon the various toxicokinetics approaches used for the

Box

Expert tools used by the fragrance industry for metabolism predictions.

- OECD QSAR Toolbox for grouping chemicals into categories; https://www.qsartoolbox.org
- Laboratory of Mathematical Chemistry OASIS *in vitro* and *in vivo* rat liver S9 metabolism simulator; http://oasis-lmc.org/products/models/metabolism-simulators/in-vitro-rat-s9-metabolism-simulator.aspx
- OASIS Tissue Metabolism Simulator (OASIS TIMES); http://oasis-lmc.org/products/software/times.aspx
- Toxic Hazard Estimation by decision tree approach (ToxTree); http://toxtree.sourceforge.net

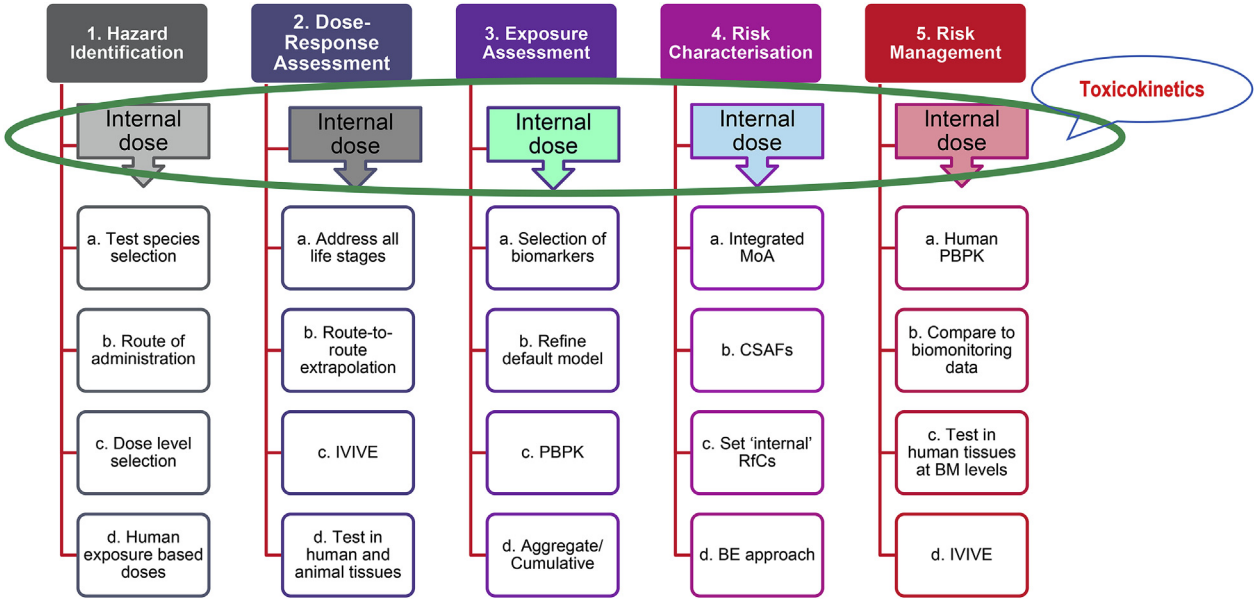

Fig. 2. AgChem toxicokinetics framework developed at Dow AgroSciences (printed with permission from Dow AgroSciences).
Abbreviations: BE = Biomonitoring equivalent; CSAF = Chemical specific adjustment factor; IVIVE = *In vitro* to *in vivo* Extrapolation; MoA = Mode-of-action; PBPK = Physiologically based pharmacokinetic model; RfC = Reference concentration.

testing of agrochemicals, a toxicokinetics guidance for this sector is being compiled within the Crop Life International project on exposure-driven human health assessment.

A case study was performed to investigate how toxicokinetic data can contribute to the hazard identification, exposure assessment and risk characterisation of agrochemicals (internal report; Dow AgroSciences). The novel herbicide halauxifen-methyl that is rapidly hydrolysed to halauxifen-acid was selected for this case study, and both compounds were assessed in 7-, 28-, and 90-day rat oral toxicity studies. After 7 days of exposure, the rats treated with halauxifen-methyl exhibited increased liver weight and liver hypertrophy, whereas no findings were recorded in the animals exposed to halauxifen-acid. Upon 28- and 90-day treatment with halauxifen-methyl, the liver was identified as target organ, and a 90-day NOAEL of 10 mg/kg body weight/day was calculated for both male and female rats. By contrast, upon 28- and 90-day treatment with halauxifen-acid, the kidneys were identified as target organ, and a much higher 90-day NOAEL of 250 mg/kg body weight/day was calculated for both male and female rats. Toxicokinetics analyses showed that the liver is exposed to halauxifen-methyl, while post-hepatic systemic exposure was only recorded for halauxifen-acid. Therefore, halauxifen-acid was selected for the subsequent long-term studies, e.g., multi-generation reproduction toxicity or carcinogenicity tests.

Within the case study, toxicokinetic data helped understand the threshold for the MoA for liver toxicity, and further allowed determination of human health reference values, e.g., the acceptable daily intake. With respect to exposure assessment, toxicokinetic data were used for PBK modelling for repeated exposures and to compare rodent data with human data thereby further improving the risk assessment.

2.3.5. Chemicals sector input

Bruno Hubesch (Hubesch Consult BVBA, Belgium), representing CEFIC LRI, summarised recent progress from relevant projects funded under this research initiative. While an abundance of *in vitro* and *in silico* models are already available, many of them require further improvements to become applicable under the REACH Regulation. One major challenge is to ensure that *in vitro* studies are performed at relevant concentrations. For this purpose, the relevant *in vitro* concentration range should be predicted from human exposure information, e.g., by traditional (forward dosimetry) PBK modelling. Conversely, it is necessary to predict equivalent human oral, dermal or inhalation exposures that are consistent with measured *in vitro* target tissue concentrations. For this purpose, reverse dosimetry PBK modelling is appropriate.

To enable reverse dosimetry modelling, the PBK model equation generator MEGen (Loizou and Hogg, 2011) from the CEFIC LRI toolbox (available at: http://cefic-lri.org/lri-toolbox/) was modified to export the models in R syntax (R Core Team, 2013) and to allow supplementing them with further user-defined models. This work is being continued within the project CEFIC-LRI-AIMT7 that aims at optimising the RVis (R Visual) as an open-access, open-source modelling platform for a biologically based, quantitative risk assessment of chemicals (*cf.* http://cefic-lri.org/projects/aimt7-rvis-open-access-pbpk-modelling-platform/). As an intuitive, user-friendly platform, the RVis shifts emphasis away from the need for specific mathematical expertise and programming skills to the toxicological knowledge underpinning chemical risk assessment. Importantly, the RVis will enable exposure predictions from *in vitro* to *in vivo* and *vice versa* and therefore also quantitative IVIVE, featuring a complete sensitivity analysis. Application of reverse dosimetry is expected to enhance the applicability of *in vitro* methods for substance risk assessment thereby contributing to the replacement of animal testing.

A further CEFIC LRI project (LRI EEM9.3-IC) aims at enhancing the predictive power of the *in silico CEFIC LRI AMBIT Tool Supporting Read-across* (https://ambitlri.ideaconsult.net/; http://ambit.sourceforge.net). AMBIT, that has been developed continuously since 2005, serves to integrate the abundance of available read-across tools. This is a challenging task due to the differences in methodologies and information technology systems applied in the different tools (QSAR models, expert systems, rule-based predictions, chemical similarity tools, etc.; *cf.* ECETOC, 2012). Further, only a limited number of the available read-across tools provides easily accessible data on substance identity and composition together with chemical structures and high-quality endpoint data. AMBIT serves as a hub to interconnect other tools and databases applying a hierarchical structure that is meaningful for hazard and risk assessment. AMBIT helps to implement workflows for read-across assessments by supporting the assessor in setting up a read-across (category) approach and in establishing a valid justification for the approach taken. This serves to ensure that all available data are used efficiently, including comparison of data between substances,

thereby minimising overall animal testing and resource costs.

Large sets of high-quality data are being processed to continuously enhance the predictive power of AMBIT. Such data are extracted from company-owned data stored in instances of the International Uniform Chemical Information Database (IUCLID), from disseminated non-confidential business information data in 14,570 dossiers of the ECHA IUCLID database, and from other reliable sources. This work has resulted in a re-launch of AMBIT2 that provides new functionalities, stand-alone and web-based versions and an open source Application Programming Interface.

AMBIT2 is compatible with the 6th version of IUCLID (https://iuclid6.echa.europa.eu), and it includes (i) the EFSA OpenFoodTox database (*cf.* Section 2.4.2 and https://www.efsa.europa.eu/en/data/chemical-hazards-data); (ii) the VEGA platform (courtesy E. Benfenati, Mario Negri Institute, Milano, Italy) that allows accessing a series of QSAR models; (iii) new additions to ToxTree (*cf.* Section 2.3.3); (iv) the Cramer classification scheme to define a TTC (Cramer et al., 1978); and (v) specific modules addressing protein binding properties. Work is under way to further improve the platform, i.e. to develop AMBIT3.

Dr. Hubesch concluded that, while AMBIT is mainly applicable for lower tier assessments, the predictions made using AMBIT are reliable so that higher tier testing needs are reduced. Finally, CEFIC LRI is continuously engaged in dissemination activities to make both the RVis and AMBIT known and readily accessible to toxicologists and risk assessors (Maertens et al., 2016).

2.4. Activities of the EC, EU agencies and multilateral research initiatives

2.4.1. Activities of the EC DG RTD

Christian Desaintes (EC DG RTD, Belgium) outlined that the EC has spent 500 million Euro since 2000 for research into toxicology, funding approximately 130 research projects. Roughly a third of this funding has been allocated to projects in the area of nanotechnologies; however, this proportion is decreasing under the Horizon 2020 Research Framework Programme. Approximately eight percent of the funding has been dedicated to projects in the area of ecotoxicology. The topics of toxicokinetics and read-across are usually integrated in larger projects. Often, new *in vitro* methods or *in silico* models are developed in the course of a project even though such development was not the main purpose of the research activity.

Future research activities on toxicology in Horizon (2020) will most likely address the topics of human hazard and risk assessment, endocrine disruption, and the human exposome. The exposome concept refers to the totality of environmental exposures from conception onwards. It is a novel approach to studying the role of the environment in human disease. Using 'omics technologies, the collected exposure data will be linked to biochemical and molecular changes in humans; *cf.* http://www.exposomicsproject.eu. One of the key topics pursued within IMI-2 (funded in equal shares by the EC and the pharmaceutical industry) is 'electronic translational safety', that encompasses the development of an internationally accepted guideline for data sharing, the collation of large sets of preclinical and high-level clinical data, and a retrospective analysis of these data to assess translation of preclinical to clinical study outcomes (*cf.* http://ec.europa.eu/research/participants/portal/desktop/en/opportunities/h2020/topics/imi2-2016-09-04.html).

Additionally, future research activities to be funded by the EC will likely be related, e.g., to nanomaterials, food safety, environmental safety, next-generation organ-on-a-chip-models, and basic research. Overall, the EC is striving to promote cross-sector collaboration, also across SMEs and between academia and industry. Similarly, it aims at linking the funded research activities to other international initiatives. A cross-sector, and also cross-project, information exchange is indispensable to streamline efforts and activities.

2.4.2. Activities of the EFSA

Jean-Lou Dorne (EFSA, Italy) summarised recent activities of EFSA that contribute to the integration of exposure, toxicokinetics, and toxicity data for risk assessment in the food safety area. Relevant publications include the *EFSA Scientific Committee Guidance on the use of the WoE approach in scientific assessments* (EFSA Scientific Committee, 2017). When integrating toxicokinetic data, the WoE evaluation should ideally address interspecies differences in ADME processes, taking into account the biological relevance of the test species for human extrapolation (e.g. differences in metabolism between humans and rats) and inter-individual toxicokinetics differences between human sub-populations (e.g. genetic polymorphism, inter-ethnic differences, children, neonates). Importantly, the WHO MoA framework, that is referred to in the EFSA guidance, includes both toxicokinetics and toxicodynamics assessments to address both interspecies and inter-individual differences (Meek et al., 2014a; b; EFSA, 2014a). Depending on the available data, uncertainties and variability of the data are reported, e.g., differences between *in vitro* and *in vivo* ADME or dosimetry differences in PBK modelling (Fig. 3).

Generally speaking, the available data entail how the risk assessment of a substance in food can be undertaken:

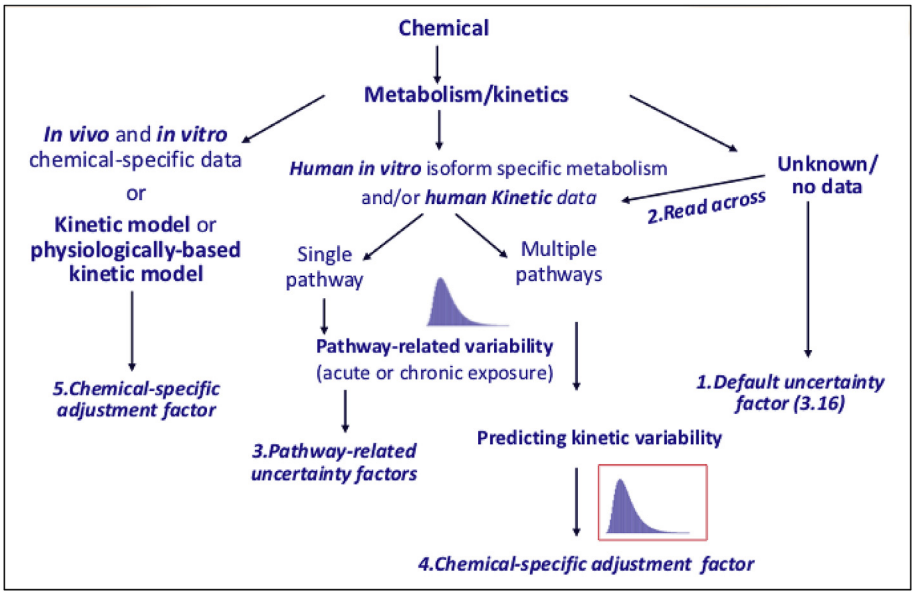

Fig. 3. Refinement of uncertainty factors allowing for human variability in toxicokinetics: Default values, pathway-related uncertainty factors, chemical-specific adjustment factors (Figure modified and reproduced with permission from Dorne (2001)).
The default uncertainty factor (UF) for human variability in toxicokinetics (3.16) applied in the absence of chemical-specific toxicokinetic data can be replaced by pathway-related variability, modelled chemical-specific adjustment factors (CSAFs), or CSAFs when chemical-specific toxicokinetic data are available (Renwick and Lazarus, 1998; Dorne et al., 2005). The rationale for splitting the default UF of 10 ($= 3.16^2$) for human variability (i.e. UF of 3.16 for both toxicokinetics and toxicodynamics) allows for incorporating chemical-specific variability in either toxicokinetics or toxicodynamics or both toxicokinetics and toxicodynamics to derive CSAFs.

1. In a data-poor scenario (e.g. emerging contaminant), the derivation of the reference point can be based on TTC or *in silico* (QSAR) data. In such cases, the reliability, relevance, and consistency of the models has to be evaluated, e.g., by statistically analysing the likelihood that a compound with a given structural alert will express toxicity. Preferably, estimates are available from different *in silico* tools. If the estimates from the different tools converge, the level of uncertainty on the predicted property can be determined taking into account the models' applicability domains. If estimates do not converge, further modelling can be necessary to improve the results.
2. For pre-market authorisation of regulated substances (agrochemicals, food additives etc.), the reference point is often derived from *in vivo* data in test species as defined in the respective regulatory requirements.
3. In a data-rich situation (e.g., re-evaluation of a contaminant or a regulated substance), the derivation of a reference point may use a full MoA analysis including PBK modelling between test species and humans, *in vivo* animal data and even epidemiological data (EFSA Scientific Committee, 2017).

Since its creation in 2002, EFSA scientific panels and staff have produced risk assessments for more than 4400 substances in over 1650 scientific opinions, statements and conclusions. In 2017, the first version of OpenFoodTox has been published as a structured database summarising the outcome of hazard characterisation for human health and, depending on the relevant legislation and intended uses, animal health and the environment (Dorne et al., 2017). For each individual substance, the OpenFoodTox data model has been designed on the basis of the OECD Harmonised Template to collect and structure the data in a harmonised manner. OpenFoodTox contains information on the identity and use of the substance, EFSA's opinion on the specific substance or groups of substances, critical toxicity studies, and health-based guidance values (such as acceptable and tolerable daily intakes). Thereby, OpenFoodTox encompasses 10,000 toxicological endpoint studies and 12,000 risk assessment summaries. OpenFoodTox can be searched under the following link using a microstrategy tool: https://dwh.efsa.europa.eu/bi/asp/Main.aspx?rwtrep=400, or it can be downloaded under EFSA's knowledge junction: https://zenodo.org/record/344883#.WUDqK_mGPIU which also contains most scientific models used by EFSA in the area of human health, animal health and environmental hazard assessment of chemicals and biologicals. The data contained in OpenFoodTox have also been used as training and test sets to develop QSAR models, e.g., to predict sub-chronic toxicity in rats (continuous NOAEL model) and lethal concentrations (LC_{50}) in rainbow trout (Toropov et al., 2017; Toropova et al., 2018). These models are available as web applications on the VEGA hub (https://www.vegahub.eu).

Further, EFSA has reviewed the applicability of available methods and tools for toxicokinetics assessments as well as 'omics technologies and *in silico* tools to investigate toxicity and toxicodynamics during human hazard assessment of chemicals (EFSA, 2014a). This review yielded a number of conclusions including the need to develop generic toxicokinetics models and tools to support food safety assessments. Since, EFSA has engaged in a multi-agency-academia collaboration to develop such generic toxicokinetics models and tools as user-friendly, open-source models, coded in R (R Core Team, 2013). The models range from simple toxicokinetics tools including allometric scaling models, one-compartment toxicokinetics models, and PBK models calibrated with physiological data for humans, farm animals, pets and species of ecological relevance. A modelling platform is under construction (TK platform) that will contain the species-specific physiological data and models, as well as metabolism data and chemical-specific data. The TK platform is expected to provide a range of applications including determination of a substance's internal dose, tissue residues, as well as interspecies differences and human variability in toxicokinetic parameters. Application of the TK platform is expected to contribute to harmonising the reporting of the sensitivity and uncertainties related to model predictions (Fig. 3). It is planned to involve stakeholders in the further development of the TK platform via an open call to share toxicokinetic data, models and case studies illustrating their applicability in food safety and other areas of risk assessment.

2.4.3. Activities of the European Medicines Agency (EMA)

Leon Van Aerts (Medicines Evaluation Board, The Netherlands), representing the EMA as member of the *CHMP Safety Working Party* and the *Biosimilar Medicinal Products Working Party*, provided an overview on ICH and EMA guidance related to pharmacokinetics and toxicokinetics. In 1995, EMA issued the *ICH Topic S3A Toxicokinetics guidance for assessing systemic exposure in toxicology studies* (EMA, 1995a). In connection with this guidance, an ICH Q&A promotes micro-sampling techniques in order to reduce the use of toxicokinetics satellite animals and sample volumes (ICH, 2017). Further, EMA issued the *ICH Topic S3B Pharmacokinetics: Repeated-dose tissue distribution studies* (EMA, 1995b). The two guidance documents on toxicokinetics and pharmacokinetics (EMA, 1995 a; b) are reiterated or further specified in:

- *ICH guideline M3 (R2) on non-clinical safety studies for the conduct of human clinical trials and marketing authorisation for pharmaceuticals* (EMA, 2009);
- *ICH harmonised tripartite guideline: Dose selection for carcinogenicity studies of pharmaceuticals S1C (R2)* (EMA, 2008);
- *ICH Topic S5 (R2/3)) Detection of toxicity to reproduction for medicinal products including toxicity to male fertility* (EMA, 1994).

The ICH – EMA guidance on toxicokinetics and pharmacokinetics provides frameworks to describe the systemic exposure achieved in animals and its relationship to the dose level and the time course of the toxicity study. Thereby, the internal exposure achieved in toxicity studies can be related to toxicological findings, which is a prerequisite for assessing the relevance of toxicity data for clinical safety assessments. Toxicokinetic data further support the choice of species and treatment regimen in non-clinical toxicity studies, and provide information which contributes to the design of subsequent non-clinical toxicity studies (*cf.* http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Safety/S3A/Step4/S3A_Guideline.pdf).

During pharmaceutical assessments, *in vitro* test methods allow investigation of metabolism (with human or animal microsomal fractions, hepatocytes, or transfected cells as test systems), protein binding, compound transport, or absorption (with Caco-2 cells or dermal constructs as test systems). Such *in vitro* data are used to predict the pharmacokinetics in subsequent clinical trials (i.e. volunteer studies in phase 1 and patient studies in phases 2 and 3). Data from *in vivo* toxicokinetics studies in animals, on the other hand, are generally needed for calculation of safety margins and thus support the safety assessment of the drug substance. It would require a paradigm shift, an entirely new approach for how to conduct all parts of the hazard and risk assessment, to allow replacement of these *in vivo* toxicokinetics studies. A comprehensive non-animal testing strategy could include predictive *in silico* tools, *in vitro* methods and IVIVE. Nevertheless, also in the present risk assessment paradigm, *in vitro* testing and *in silico* modelling, integrated into read-across approaches, can serve to reduce endpoint-specific *in vivo* testing for specific substances or groups of substances.

2.4.4. Activities within the EU project EuroMix

Vikas Kumar (University of Rovira i Virgili, Spain) presented activities from the ongoing Horizon 2020-funded project *EuroMix – European Test and Risk Assessment Strategies for Mixtures* (https://www.euromixproject.eu). It is the overall objective of EuroMix to develop a tiered testing strategy for the risk assessment of mixtures, containing multiple substances derived from multiple sources across different life stages of the substances (production, use, disposal), and covering

exposure assessment via multiple exposure routes. This mechanism-based testing strategy makes use of QSAR modelling and the TTC approach to prioritise the substances under investigation (Tier 1). In Tier 2, large numbers of substances and mixtures are assessed in a bioassay tool box, and, in Tier 3, selected substances are evaluated in *in vivo* studies, if necessary. PBK models, embedded in an online integrated modelling platform, are used for IVIVE and to identify pathways and biomarkers of relevance in humans. In this new paradigm of integrated hazard and risk assessment, MoAs and dose-response assessments play an important role (Sharma et al., 2017a). The integrated framework will enhance the understanding on risks not only based on target tissue concentrations, but also on the effect on the target molecule participating in the given biological network (Sharma et al., 2017a). Following this mechanism-based testing strategy, EuroMix has developed a refined grouping strategy for cumulative assessment groups of agrochemicals (EFSA, 2014b) that integrates QSAR modelling and the TTC approach to conclude on the similarity or dissimilarity of MoAs of different substances.

To further develop this testing strategy, EuroMix is engaged in developing and validating *in silico* and *in vitro* tools, focussing on three selected endpoints, i.e. hepatotoxicity (liver steatosis), endocrine disruption (oestrogen/androgen balance), and developmental toxicity (skeletal malformation and cleft palate). PBK models reflecting a compartmentalised structure are being developed, where compartments represent target organs and are based on physiological tissue volumes (Fig. 1). These PBK models further include a mechanism-based description of substance biodistribution using tissue blood flow and simulations of *in vivo* transport processes. Risk assessment for specific sub-populations, such as foetuses, is one of the areas where PBK modelling can be extended to predict risks based on dosimetry, thereby combating the challenge of inaccessible data (Martinez et al., 2017; Sharma et al., 2018). Case studies are underway to assess these PBK models using animal data from the joint *Food and Agriculture Organisation/World Health Organisation Joint Meeting on Pesticide Residues* monographs on PPPs (*cf.* http://www.who.int/foodsafety/areas_work/chemical-risks/jmpr/en/) and other regulatory data.

Remaining challenges in the further development of *in silico* models include the need to establish parameter databases for human PBK models and to improve the available QSAR models. Further, PBK models need to be linked with other tools, such as AOP and mechanistic models, and system biology via key intermediates and molecular initiating events, that enable systematic understanding of the toxicity of the substance (Sharma et al., 2017b). Finally, databases on cell response systems are required that include information on dose-response relationships, as well as functional genomic tools that allow mapping and modelling pathways.

2.4.5. Activities within the EU-project EU-ToxRisk

Iain Gardner (SIMCYP, United Kingdom) presented activities from the Horizon 2020-funded project EU-ToxRisk (http://www.eu-toxrisk.eu) that are related to the use of IVIVE and PBK modelling. It is the vision of EU-ToxRisk to drive the paradigm shift in toxicological testing away from animal testing towards assessments based on human cell responses and a comprehensive mechanistic understanding of cause-consequence relationships of substance adverse effects. For this purpose, EU-ToxRisk is integrating advancements in cell biology, 'omics technologies, systems biology and computational modelling to define the chains of events that link substance exposure to toxic outcome.

Exemplarily, PBK models have been used to assess internal exposure to a variety of substances including drugs. These PBK models include estimates of absorption rates and extent, metabolism, distribution into tissues, the excretion of the unchanged drug, and drug transport to predict drug concentration and effect in humans. IVIVE techniques are used to provide the input parameters for the PBK models. Data obtained with human liver microsomes, fresh or cryopreserved human hepatocytes, or recombinantly expressed human enzymes are used to predict *in vivo* metabolic drug clearance (Fisher et al., 2016). Hepatic scaling factors are required for IVIVE to convert the measured rate of metabolism in the *in vitro* system to an *in vivo* rate per whole liver. To describe the disposition of the compounds in a target population, the relevant distributions of values for demographical, biological, physiological and genetic parameters are identified, and the covariation between these parameters is assessed.

Case studies assessing different calcium channel blockers that are predominantly cleared by cytochrome P450 3A4 metabolism yielded good concordance between the observed and predicted blood clearance upon oral or intravenous administration, and the observed and predicted apparent volume of drug distribution at steady state (Vss). Predictions for structurally unrelated cytochrome P3A4 substrates were also satisfactory (up to 3-fold differences between observed and predicted data). These case studies confirmed that PBK approaches using human *in vitro* and physico-chemical data and IVIVE can be combined to predict substance concentrations in the body for a series of structurally related substances. Some challenges remain to be addressed to allow use of IVIVE methods for non-pharmaceutical substances, and to allow adapting predictions to different routes of exposure (oral versus

Table 2
Sectorial use of PBK modelling.

Sector	Use of PBK modelling
Pharmaceuticals and vaccines	Within pharmaceutical (in particular small molecule) drug discovery and development, there has been a significant increase in the use of PBPK modelling in support of internal decision making as well as in the acceptance by regulatory health authorities in support of clinical development and ultimately drug label claims. The majority of these PBPK-based success stories are related to drug-drug interactions and an extensive data package based on *in vitro*, animal, as well as clinical data is often required in order to obtain regulatory acceptance (Shebley et al., 2018).
Cosmetics	In some cases, PBK modelling is being used for risk assessment purposes. The Scientific Committee for Consumer Safety (SCCS) considers all available scientific data including data from PBK modelling for the safety evaluation of cosmetic substances. In its Notes of Guidance for the Testing of Cosmetic Ingredients and Their Safety Evaluation (SCCS, 2015), the SCCS defined the conditions for the use of PBK models submitted for risk assessment purposes.
Fragrances	A dermal model for skin absorption of fragrance materials is widely used (Shen et al., 2014), but routine use of PBK considerations is not common yet for the risk assessment of fragrance ingredients.
Agrochemicals	Not frequently, but PBK modelling has been used for various purposes e.g. understanding toxicokinetics differences between various species and therefore potentially refining the default uncertainty factors of 100x; understanding potential internal exposures in humans for risk assessments; deriving human Biomonitoring Guidance Values (Arnold et al., 2015), etc. As no human toxicokinetics data can be generated for new agrochemicals (biomonitoring data can only be available post-marketing), validation of PBK models is one of the major challenges.
Chemicals	PBK modelling is not yet widely used for regulatory purposes. In accordance with the REACH Regulation (EP and Council, 2006), the toxicokinetic behaviour of a substance generally has to be assessed to the extent that can be derived from available information.
Food safety	In the food safety area, a number of models are available mostly for agrochemicals and food contaminants addressing effects in humans, laboratory animal species and species of ecological relevance (e.g. fish). However, toxicokinetic data and PBK modelling are still hardly used for food safety assessments. One of the reasons for this is the lack of internationally accepted guidance on the use of toxicokinetic data for risk assessment, ranging from basic parameters to full PBK models.

dermal versus inhalation).

3. Discussion

3.1. Applicability of the available PBK models and hurdles preventing their regulatory use

In a number of industry sectors, different PBK models are already being used during different steps of hazard and risk assessment. However, in other industry sectors, PBK modelling is rarely used for regulatory purposes (Table 2). An important hurdle to PBK modelling is the lack of available toxicokinetic data in different animal species and humans for which reason the models often rely on *in vitro* data inputs or *in silico* predictions. The availability of animal and human toxicokinetic data also depends upon the legal data requirements implemented for the respective sector.

PBK modelling in the pharmaceutical sector benefits from the circumstance that human data are generally available for pharmaceutical substances, which is rarely the case in the other sectors (exceptions are, e.g., certain data from cosmetovigilance). Therefore, it is much more difficult to assess the human relevance of *in silico* predictions in the non-pharmaceutical sectors. Nevertheless, pharmaceuticals can serve as reference substances for PBK modelling that extends beyond the pharmaceutical sector.

In the agrochemicals sector, data from rat toxicokinetics studies (OECD TG 417; OECD, 2010), that are part of the standard information requirements, are helpful for toxicokinetics evaluations. Hence, in this industry sector, *in vivo* toxicokinetic data are generally available to substantiate the modelling, whereas other input parameters, including human data, are generally lacking. For the chemicals sector, *in vivo* toxicokinetic data or other input parameters are generally rare: In accordance with the REACH Regulation (EP and Council, 2006), the toxicokinetic behaviour of a substance generally has to be assessed to the extent that can be derived from available information.

In the veterinary pharmaceutical or vaccines sectors, substance-induced effects have to be assessed in target animals and often also in toxicological species (rat, dog) for assessment of human safety (as relevant to manufacturing, user and human food safety of veterinary pharmaceuticals). The value of including such data into the databases for PBK modelling merits further evaluation. Data from target animals could serve to reveal species-specific physiological or pathological differences. Even though it might be laborious to comprehensively cover the spectrum of known species-specific differences in PBK models, their inclusion may improve the predictivity of the modelling.

In the food safety area, a number of models are available mostly for agrochemicals and food contaminants (including persistent organic pollutants, metals, perfluoralkyls, etc.) addressing effects in humans, laboratory animal species and species of ecological relevance (e.g. fish). However, toxicokinetic data and PBK modelling are still hardly used for food safety assessments. One of the reasons for this is the lack of internationally accepted guidance on the use of toxicokinetic data for risk assessment, ranging from basic parameters to full PBK models. In the near future, EFSA is planning to initiate the development of such a guidance with a broad scope.

A number of *in silico* toxicokinetics tools are already available that allow good predictions of specific ADME parameters. Since most toxicokinetics tools have been developed mostly using pharmaceutical substances, they yield satisfactory results for sufficiently similar small molecules, such as a number of agrochemicals, contaminants and food additives. By contrast, for more dissimilar, larger molecules, including polymers, high-quality data to corroborate the *in silico* models are mostly unavailable, and economic restraints can prevent the further development of the modelling tools for such applications. Since PBK modelling (just as *in vitro* toxicokinetics methods) continues to be complex and resource intensive (i.e. it requires specific expertise to run them), it has to be determined in advance which specific information is necessary and relevant during which step of the hazard and risk assessment.

While PBK modelling is useful to predict if a specific substance is metabolised into a specific metabolite, it is still difficult to jointly model or even quantify the presence of an abundance of different metabolites in all relevant body fluids. Even if such modelling was possible, it is currently unclear how such information could be incorporated into human risk assessments. Furthermore, the detoxification of a substance may be difficult to predict. Next to metabolism, the presence and activity of different transporters in various tissues and the potential for interactions at this level are complicating factors affecting tissue concentrations. To date, such specific information has only been incorporated into PBK models to a limited extent, where, usually, kinetic parameters are globally described at tissue levels.

A number of uncertainties can affect the reliability and reproducibility of PBK modelling, including chemical uncertainties and data variability (Fig. 3). Since it can be difficult to reproduce the data from commercially available tools, guidance is under development by the OECD on how the variability and uncertainty of such tools should be addressed to facilitate their regulatory use in the chemical and agrochemical sectors. A tripartite dialogue between model developers, users, and regulators is advisable to agree on the benefits and uncertainties involved with the application of a new *in silico* tool. Case studies can serve to provide relevant data to underpin such evaluations. Transparency and clear guidance are required with respect to the application of a new PBK model to understand the relevance and reliability of the resulting predictions. Further, the relevance of the specific parameters encompassed in a specific model may have to be re-assessed at regular intervals.

Even though *in silico* models can also be developed for specific types of substances, the major initiatives aim at building generic PBK models. Collaboration between different companies, across sectors and between industry and the relevant public authorities are key to the successful outcome of such efforts. Generally, there is no competitive advantage to be gained by developing *in silico* models for in-house use alone since the predictivity of the models is likely to increase the more data from different sources are used. Furthermore, for the ultimate goal of regulatory acceptance, new models have to be transparent as far as possible. One obstacle may be sharing of proprietary data that are relevant for *in silico* modelling. To enable their use, a data sharing agreement can be established via the 'broker bridge approach' by which the data are stored with an independent third party (Long et al., 2013). Thereby, the proprietary information is kept confidential, while the tools as such are available for wider use.

3.2. 3Rs impact of in vitro and in silico toxicokinetics models

The extent to which *in vitro* methods and *in silico* models serve to replace *in vivo* toxicokinetics studies is also dependent upon the legal provisions that are relevant for the given industrial sector. For the cosmetics sector, a general animal testing ban and a ban to market cosmetic ingredients tested on animals is implemented in the EU under the Cosmetic Products Regulation (EP and Council, 2009b). Therefore, new testing for the assessment of cosmetic ingredients must rely solely on non-animal methods. Under REACH, *in vivo* toxicokinetics studies are generally not conducted, whereas they may have to be performed in the pharmaceutical and agrochemicals sectors where data on toxicokinetic properties are requested (*cf.* Section 3.1).

While a number of *in vitro* models are available to predict mostly local effects upon single exposure, there are no standardised or validated *in vitro* models reflecting repeated-dose effects, especially for systemic toxicity. When relying on *in vitro* and *in silico* data alone to qualify, or even quantify ADME parameters, a certain level of uncertainty has to be accepted, as it is currently the case for *in vivo* data. Since it is difficult to define uncertainty as such, it is also difficult to define which level of uncertainty, and hence risk, to consider

acceptable. This applies in equal manner to the animal studies, that also serve to predict effects in humans (As explained in Section 2.3.1, for PBPK modelling, the current aim is to obtain a less than three to four-fold difference between predicted and observed human exposure data.).

Confidence in any new (*in vitro* or *in silico*) methodology for regulatory decision-making is increased once it has been successfully validated. If high-quality human data are unavailable for the validation exercise (from studies in volunteers and patients, epidemiological reports or cosmetovigilance, etc., as applicable for the given sector), the *in vitro* or *in silico* methodologies have to be validated against animal data. Possible inherent limitations of the animal data can affect the outcomes of such validation studies. Specifically, toxicokinetic data obtained in rodents may poorly predict the kinetics in humans (Wood et al., 2017; Horiuchi et al., 2018). Confidence in the reliability of *in vitro* data is increased if the (patho-)physiological events reflected by the given *in vitro* test system are clearly defined (e.g., the cytochrome P450 enzyme system (Donato and Castell, 2003)). The predictivity of *in vitro* methods can be improved by combining them into testing batteries, or by integrating the data from such methods into more complex *in silico* tools. Whenever *in silico* tools are solely based on *in vitro* ADME data, the more comprehensive the physico-chemical data on the substance under investigation is then the greater the robustness of the modelling.

3.3. Use of in vitro and in silico toxicokinetics to support read-across

The relevance of (the type of) toxicokinetic and pharmacokinetic data in support of read-across has to be determined on a case-by-case basis (further taking into account the validity of the applied tools; *cf.* Section 3.2). In many cases, toxicokinetics and pharmacokinetics data are likely to be of substantial relevance for read-across, since they serve to broaden the understanding of the effects that the target substance can elicit in humans or the environment. While the read-across approach is generally based on the structural similarity between the source substance(s) and the target substance, information on ADME, and metabolism in particular, enhances the robustness of the read-across by deepening the mechanistic understanding of endpoint-specific effects (Aggarwal et al., 2014, 2015; Hand et al., 2017). This serves to increase the confidence in the conclusion from the read-across approach. Additionally, IVIVE considerations can be useful to substantiate the read-across justification.

The better the chemical analogue or chemical category is defined, the more likely it is that the read-across conclusion on the property under investigation will be accepted. When using toxicokinetic data in support of read-across, it is beneficial if toxicokinetic data are available both for (at least some of) the source substance(s) and the target substance. This will enable cross-comparisons of both the given endpoint-specific effects and the toxicokinetics properties. If *in vivo* or *in vitro* toxicokinetic data are unavailable for the source substance(s), the missing toxicokinetic data should be obtained applying relevant *in silico* tools. In such cases, the *in silico* predictions of toxicokinetic properties for the source substance(s) and the target substance should preferably be obtained in the same models, thereby improving data comparability.

Attempts to use PBK model predictions and read-across approaches to support risk assessment have been published by Alajlouni et al. (2016) and Al-Malahmeh et al. (2017), who applied model predictions to support read-across from one substance for which sufficient information on tumour data were available to a second substance for which limited *in vivo* data were available, thereby facilitating a preliminary risk assessment. These studies showed how PBK modelling can facilitate read-across from compounds for which *in vivo* toxicity studies on a specific endpoint are available to compounds for which these data are unavailable (Alajlouni et al., 2016).

The ECHA publication *Grouping of substances and read-across approach* (ECHA, 2013) exemplifies how toxicokinetic data, and specifically PBK modelling, can enhance the regulatory acceptance of read-across: *A registrant claims (i.e. hypothesis) that the target substance is rapidly hydrolysed to the source substance following oral administration because the target substance is believed to decompose in the low* pH *of the stomach. Without supporting data to substantiate the hypothesis, the read-across cannot be accepted. On the other hand, supporting information (e.g. experimental studies on hydrolysis at gastric* pH*, combined with absorption data and PBPK modelling) contributes to increasing the reliability of the read-across approach* (ECHA, 2013, p. 8).

3.4. The applicability of read-across approaches for regulatory hazard and risk assessment

While most industry sectors use read-across approaches to some extent during R&D, the regulatory use of read-across approaches differs between sectors. Read-across approaches are scarcely used to meet the information requirements for PPPs or BPs (Aggarwal et al., 2014). Under REACH, read-across approaches were used to fulfil the information requirements for at least one endpoint in 75% of the submitted registration dossiers that the ECHA analysed by 2014 (ECHA, 2016). Nevertheless, the application of read-across remains challenging for complex toxicological endpoints (ECHA, 2017b). Further, the ECHA concluded that adaptations based on read-across and WoE are often poorly documented and justified, and therefore not acceptable (ECHA, 2016). On the other hand, registrants have expressed the wish to improve transparency on the specific reasons behind the ECHA's decisions on the non-acceptance of read-across approaches (Ramirez et al., 2015).

The following activities are expected to enhance the scientific robustness of read-across tools thereby facilitating their regulatory use and acceptance:

- Collation of major read-across tools used in the different industry sectors including an evaluation of their strengths and weaknesses;
- Continuation of initiatives to combine read-across tools in a user-friendly manner;
- Expansion of the major read-across tools to enable automatic and user-friendly documentation and justification of the read-across (and of outputs from *in silico* tools applied in the course of the read-across);
- Review to identify areas (in terms of toxicological endpoints, products, etc.) in which read-across approaches are applied successfully, and to reveal knowledge gaps preventing such successful use;
- Formal guidance by the responsible regulatory authority specifying the minimum information required to justify the application of read-across for a given toxicity endpoint.

While read-across approaches are currently founded on structural similarity (ECHA, 2017a), it should be further explored if similarities in biological activity might also become a starting point for read-across. While such an approach would require a paradigm shift with respect to the grouping of substances and the application of read-across techniques, it may improve the human relevance of read-across predictions. Toxicokinetics elements are a crucial pre-requisite for such an approach.

The following activities are expected to facilitate the use of *in vitro* and *in silico* toxicokinetic data in support of read-across approaches in a regulatory setting:

- Collation of *in vitro* and *in silico* toxicokinetics tools, further indicating which specific ADME parameters they address and which chemical applicability domain they cover;
- Endpoint-specific guidance on toxicokinetics parameters that are required for read-across;
- Case studies to exemplify how toxicokinetic data help support the scientific robustness of read-across.

A cross-sector coordination of the ongoing initiatives on *in vitro* and

in silico toxicokinetics assessments in support of read-across will enhance the applicability of these tools. Confidence in applying read-across for regulatory purposes and in using *in vitro* and *in silico* toxicokinetic data in support of read-across will increase as further experience is gained in applying these tools, both on the side of industry and authorities. A continuous dialogue between the developers of toxicokinetics and read-across tools, users and regulators is indispensable to ensure that all aspects that are pivotal to promote the regulatory acceptance and use of read-across and *in vitro* and *in silico* toxicokinetic data are adequately addressed. Preferably, such cooperation should take place both on the EU level and the international level.

Further dissemination activities are advisable to inform on the animal welfare and economic benefits of read-across and the consideration of *in vitro* and *in silico* toxicokinetic data in support of read-across. Toxicokinetics that are relevant for the understanding of metabolism help improve the scientific robustness of read-across. Therefore, use of *in vitro* and *in silico* toxicokinetic data in support of read-across can help improve the regulatory acceptance of such approaches.

Regulators require trust that the underlying tools are sufficiently valid. On the other hand, industry requires assurance that the predictions will be accepted once a pre-defined set of information is submitted. Validation efforts are necessary to ensure that *in vitro* and *in silico* predictions are accepted as replacements to animal testing and not only as supplements. Once *in vitro* methods and *in silico* tools have been assessed as valid, both negative and positive predictions should be accepted for regulatory purposes. On the longer-term, meeting this goal will also require adaptation of relevant legislation and guidance.

4. Conclusions and recommendations

As an outcome of the EPAA Partners' Forum *Finding synergies for* 3Rs – *toxicokinetics and read-across*, the following conclusions were made:

- Toxicokinetic data play an important role in risk assessment across all industry sectors and are subject to significant research effort by all relevant stakeholders.
- Toxicokinetic data (*in vivo* data; ADME) strengthen the read-across WoE.
- Toxicokinetic data should ideally be anchored towards human relevance and preferably be generated using *in vitro* methods and *in silico* tools.
- Identification and characterisation of metabolism is a key component of toxicokinetics assessment in read-across.

The participants of the Forum expressed confidence that significantly more progress in toxicokinetics can be made if collaboration between sectors is enhanced and synergies are captured. In this context, the following actions were recommended:

1. Promote the establishment of a Toxicokinetics Speciality Section within EUROTOX;
2. Explore possibilities to create a database of modern toxicokinetic tools to assist hazard and risk assessment, building on existing information (e.g., EFSA, 2014a); collation of a contemporaneous list of open tools (open source, managed by the EPAA);
3. Dissemination and training event on RVis with toxicokinetics stakeholders in early 2019.

In closing the EPAA Partners' Forum *Finding synergies for* 3Rs – *toxicokinetics and read-across*, *Renate Weissenhorn (EC DG GROW),* the EPAA EC Co-Chair, expressed her appreciation that the Partners' Forum had fully met all of its aims: The fruitful discussions had served to identify synergies and to provide incentives for future cross-sector collaboration. Experience in developing and applying *in vitro* and *in silico* toxicokinetics tools and read-across had been shared between different stakeholders, and this had served to enhance the networking between experts in the field. The established contacts would be beneficial for further initiatives to promote the development and use of non-animal testing methodologies for regulatory purposes. This successful outcome highlighted the unique role of the EPAA as neutral platform operated on equal terms by the EC and companies and trade associations from eight industry sectors.

5. Glossary

3Rs: Replacement, reduction and refinement of animal testing, as defined for the first time in 1959 in *The Principles of Humane Experimental Technique* (Russell and Burch, 1959).

ADME: Absorption, distribution, metabolism, and elimination/excretion.

Adverse outcome pathway (AOP): A linear sequence of events commencing with initial interaction(s) of a stressor with a biomolecule within an organism that causes a perturbation in its biology (i.e., molecular initiating event), which can progress through a dependent series of intermediate key events and culminate in an adverse outcome considered relevant to risk assessment or regulatory decision-making (Ankley et al., 2010; OECD, 2013, 2017b). In contrast to modes-of-action (MoAs; *cf.* definition), AOPs are not substance-specific and therefore do not include metabolism considerations. AOPs can help address the biological plausibility of a MoA (ECETOC, 2017).

External dose: In an *in vivo* study, the applied (nominal) dose (*cf.* also definition for internal dose).

Grouping (of chemicals): The general approach for considering more than one chemical at the same time. It can include formation of a chemical category or identification of chemical analogue(s) with the aim of filling data gaps as appropriate (OECD, 2014).

***In vitro* to *in vivo* extrapolation (IVIVE):** A process of using *in vitro* data to predict *in vivo* kinetics and thereby estimate exposures that could be associated with adverse effects (Punt et al., 2011; Chang et al., 2015; Wambaugh et al., 2018).

Integrated approach for testing and assessment (IATA): A pragmatic, science-based approach for chemical hazard characterisation that relies on an integrated analysis of existing information coupled with the generation of new information using testing strategies. IATA follow an iterative approach to answer a defined question in a specific regulatory context, taking into account the acceptable level of uncertainty associated with the decision context (OECD, 2016b).

Internal dose: In an *in vivo* study, the dose that reaches a specific organ or body tissue (*cf.* also definition for external dose) (DeWoskin et al., 2013).

Kinetics: The time- and concentration-dependent fate of a substance in a biological system (e.g., body, cell) in dependence of its absorption, distribution, metabolism, and elimination/excretion (ADME) rate and extent (adapted from Bessems et al., 2015).

Mode-of-action (MoA): "*A biologically plausible sequence of key events leading to an observed effect supported by robust experimental observations and mechanistic data. A MoA describes key cytological and biochemical events – that is, those that are both measurable and necessary to the observed effect – in a logical framework*" (WHO, 2009; Definitions page A-25).

Pathway (biological or biochemical): A series of molecular events occurring in a cell (or extracellularly) that leads to a certain product or an intra- or extracellular alteration. A pathway can, for example, trigger the assembly of new molecules, and will thus include direct substance-target interactions, cellular signalling and cellular regulatory processes (Buesen et al., 2017; adapted from: US National Human Genome Research Institute; *cf.* https://www.genome.gov/27530687/biological-pathways-fact-sheet/).

Pharmacokinetics: The science of describing the disposition (concentration versus time) profile of a substance within the body. Mainly focuses on describing and modelling the kinetics in a pharmaceutical substance's therapeutic range (*cf.* also definition for kinetics).

Physiologically-based kinetic (PBK) modelling: Modelling to describe the fate of a substance in the organism by mathematical equations. PBK is a more general term for specific ones used so far, such as PBPK (physiologically-based pharmacokinetic), PBBK (physiologically-based biokinetic), or PBTK (physiologically-based toxicokinetic), the latter term considered inappropriate since it is the dose a substance, and not the kinetics, that determine toxicity (Clewell et al., 2008). In the current report, 'PBK' is used as standard term, referring to 'PBPK' in case the modelling exclusively relates to pharmacokinetics (*cf.* definition).

Read-across: Under *Regulation (EC) No 1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH)*, read-across is a technique for predicting endpoint information for the target substance by using available data from the same endpoint from the source substance(s) (adapted from ECHA, 2017a).

Read-across approach: Encompasses (i) elements addressing the structural similarity; (ii) a read-across hypothesis; (iii) a read-across justification; and (iv) the prediction of the property (properties) of the target substance(s).

- The **analogue approach** is employed between a few, very structurally similar substances for which it is not possible to establish a trend or a regular pattern.
- The **category approach** is employed between several substances that are grouped together based on defined structural similarity for one or more (toxicological or other) properties. Predictions are made within the group for the target substance(s) based on the observed regular pattern (adapted from ECHA, 2017a).

Reverse dosimetry: Extrapolation of a dose-response relationship *in vitro* to *in vivo* by using PBK modelling (Chang et al., 2015).

Threshold of toxicological concern (TTC): The TTC concept uses distributions of no-observed-adverse-effect levels (NOAELs) from available *in vivo* toxicity studies, dividing the 5th percentile value by an uncertainty factor to derive the generic human exposure TTC value (Munro et al., 1996; Kroes et al., 2000; EFSA Scientific Committee, 2012; Laufersweiler et al., 2012; Partosch et al., 2015).

Toxicodynamics: Describes the interaction of substances with biological targets and how this may lead to adverse health effects (adapted from Bessems et al., 2015).

Toxicokinetics: *The quantitative study of the movement of an exogenous chemical from its entry into the body, through its distribution to organs and tissues via the blood circulation, and to its final disposition by way of biotransformation and excretion*' (Klaasen et al., 2013). Similarly to pharmacokinetics, toxicokinetics describes and models the kinetics of a substance, but into the toxic range of effects, which are usually identified in standard toxicity studies (*cf.* also definitions for pharmacokinetics and kinetics). Information on toxicokinetics also helps to refine risk assessments, when *in vitro* to *in vivo* extrapolations are concerned (*cf.* e.g. Bosgra and Westerhout, 2015).

Uncertainty: A general term referring to all types of limitations in available knowledge that affect the range and probability of possible answers to an assessment question (EFSA Scientific Committee, 2017).

Weight-of-evidence (WoE): The extent to which evidence supports one or more possible answers to a scientific question. Hence, a WoE assessment is a process in which all available evidence is integrated to determine the relative support for possible answers to a scientific question (EFSA Scientific Committee, 2017). WoE is a comprehensive, integrated, often qualitative judgment of the extent and quality of information supporting a hypothesis for which the approaches and tools vary, depending on the context (Weed, 2005; WHO-UNEP, 2012).

Conflicts of interest

The authors of this article participated in the workshop that was organised by the EPAA. Some of the authors received reimbursement of their travel expenses by the EPAA to make their participation in the workshop possible. If deemed necessary, a list of those people who received travel expenses support can be provided. UGS was hired by the EPAA to assist in the preparation of the manuscript. The other authors were engaged in the course of their normal employment. The authors alone are responsible for the content and writing of the paper.

Disclaimer

The views expressed in this manuscript by staff members/officials of the European Commission, European Agencies or other regulatory bodies are those of the individual author(s) and do not necessarily represent/reflect the views and policies of their organisation. Likewise, the opinions expressed and arguments employed herein are those of the authors and do not necessarily reflect the official views of the OECD or of the governments of its member countries.

Work of Dr. Kumar (URV) has been supported by EU's project EuroMix (European Test and Risk Assessment Strategies for Mixtures) by the EU's Horizon 2020 Research and Innovation Programme under grant agreement no. 633172.

Dr. Gardner who presented results from the EU-TOXRisk project is an employee of Certara-Simcyp. This EU-TOXRisk project has received funding from the EU's Horizon 2020 Research and Innovation Programme under grant agreement No 681002. The views expressed by Dr. Gardner reflect only the author's view and the European Commission is not responsible for any use that may be made of the information presented.

Acknowledgements

The authors thank Dr. Jean-Lou Dorne for presenting EFSA's activities in the field at the EPAA Partners' Forum and for his input in the manuscript.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.yrtph.2018.08.006.

Transparency document

Transparency document related to this article can be found online at https://doi.org/10.1016/j.yrtph.2018.08.006.

References

Aggarwal, M., Battalora, M., Fisher, P., Hüser, A., Parr-Dobrzanski, R., Soufi, M., Mostert, V., Strupp, C., Whalley, P., Wiemann, C., Billington, R., 2014. Assessment of *in vitro* human dermal absorption studies on pesticides to determine default values, opportunities for read-across and influence of dilution on absorption. Regul. Toxicol. Pharmacol. 68 (3), 412–423.

Aggarwal, M., Fisher, P., Hüser, A., Kluxen, F.M., Parr-Dobrzanski, R., Soufi, M., Strupp, C., Wiemann, C., Billington, R., 2015. Assessment of an extended dataset of *in vitro* human dermal absorption studies on pesticides to determine default values, opportunities for read-across and influence of dilution on absorption. Regul. Toxicol. Pharmacol. 72 (1), 58–70.

Al-Malahmeh, A.J., Al-Ajlouni, A., Wesseling, S., Soffers, A.E., Al-Subeihi, A., Kiwamoto, R., Vervoort, J., Rietjens, I.M., 2017. Physiologically based kinetic modeling of the bioactivation of myristicin. Arch. Toxicol. 91 (2), 713–734.

Alajlouni, A.M., Al Malahmeh, A.J., Kiwamoto, R., Wesseling, S., Soffers, A.E., Al-Subeihi, A.A., Vervoort, J., Rietjens, I.M., 2016. Mode of action based risk assessment of the botanical food-borne alkenylbenzene apiol from parsley using physiologically based kinetic (PBK) modelling and read-across from safrole. Food Chem. Toxicol. 89, 138–150.

Ankley, G.T., Bennett, R.S., Erickson, R.J., Hoff, D.J., Hornung, M.W., Johnson, R.D., Mount, D.R., Nichols, J.W., Russom, C.L., Schmieder, P.K., Serrrano, J.A., Tietge, J.E., Villeneuve, D.L., 2010. Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment. Environ. Toxicol. Chem. 29, 730–741.

Api, A.M., Belsito, D., Bruze, M., Cadby, P., Calow, P., Dagli, M.L., Dekant, W., Ellis, G., Fryer, A.D., Fukayama, M., Griem, P., Hickey, C., Kromidas, L., Lalko, J.F., Liebler,

D.C., Miyachi, Y., Politano, V.T., Renskers, K., Ritacco, G., Salvito, D., Schultz, T.W., Sipes, I.G., Smith, B., Vitale, D., Wilcox, D.K., 2015. Criteria for the research Institute for fragrance materials, inc. (RIFM) safety evaluation process for fragrance ingredients. Food Chem. Toxicol. 82 (Suppl. l), S1–S19.

Arnold, S.M., Morrissm, A., Velovitchm, J., Jubergm, D., Burnsm, C.J., Bartelsm, M., Aggarwalm, M., Poetm, T., Haysm, S., Pricem, P., 2015. Derivation of human Biomonitoring Guidance Values for chlorpyrifos using a physiologically based pharmacokinetic and pharmacodynamic model of cholinesterase inhibition. Regul. Toxicol. Pharmacol. 71 (2), 235–243.

Ball, N., Cronin, M.T., Shen, J., Blackburn, K., Booth, E.D., Bouhifd, M., Donley, E., Egnash, L., Hastings, C., Juberg, D.R., Kleensang, A., Kleinstreuer, N., Kroese, E.D., Lee, A.C., Luechtefeld, T., Maertens, A., Marty, S., Naciff, J.M., Palmer, J., Pamies, D., Penman, M., Richarz, A.N., Russo, D.P., Stuard, S.B., Patlewicz, G., van Ravenzwaay, B., Wu, S., Zhu, H., Hartung, T., 2016. Toward good read-across practice (GRAP) guidance. ALTEX 33 (2), 149–166.

Berggren, E., Amcoff, P., Benigni, R., Blackburn, K., Carney, E., Cronin, M., Deluyker, H., Gautier, F., Judson, R.S., Kass, G.E., Keller, D., Knight, D., Lilienblum, W., Mahony, C., Rusyn, I., Schultz, T., Schwarz, M., Schüürmann, G., White, A., Burton, J., Lostia, A.M., Munn, S., Worth, A., 2015. Chemical safety assessment using read-across: assessing the use of novel testing methods to strengthen the evidence base for decision making. Environ. Health Perspect. 123 (12), 1232–1240.

Berggren, E., White, A., Ouedraogo, G., Paini, A., Richarz, A.N., Bois, F.Y., Exner, T., Leite, S., Grunsven, L.A.V., Worth, A., Mahony, C., 2017. Ab initio chemical safety assessment: a workflow based on exposure considerations and non-animal methods. Comput. Toxicol. 4, 31–44.

Bessems, J., Coecke, S., Gouliarmou, V., Whelan, M., Worth, A., 2015. EURL ECVAM Strategy for Achieving 3Rs Impact in the Assessment of Toxicokinetics and Systemic Toxicity. JRC Science and Policy Report. EUR 27315 EN. available at. http://publications.jrc.ec.europa.eu/repository/bitstream/JRC96418/eurl%20ecvam%20toxicokinetics%20strategy.pdf.

Bessems, J.G.M., Paini, A., Gajewska, M., Worth, A., 2017. The margin of internal exposure (MOIE) concept for dermal risk assessment based on oral toxicity data - a case study with caffeine. Toxicology 392, 119–129.

Bosgra, S., Westerhout, J., 2015. Interpreting *in vitro* developmental toxicity test battery results: the consideration of toxicokinetics. Reprod. Toxicol. 55, 73–80.

Buesen, R., Chorley, B.N., da Silva Lima, B., Daston, G., Deferme, L., Ebbels, T., Gant, T.W., Goetz, A., Greally, J., Gribaldo, L., Hackermüller, J., Hubesch, B., Jennen, D., Johnson, K., Kanno, J., Kauffmann, H.M., Laffont, M., McMullen, P., Meehan, R., Pemberton, M., Perdichizzi, S., Piersma, A.H., Sauer, U.G., Schmidt, K., Seitz, H., Sumida, K., Tollefsen, K.E., Tong, W., Tralau, T., van Ravenzwaay, B., Weber, R.J.M., Worth, A., Yauk, C., Poole, A., 2017. Applying 'omics technologies in chemicals risk assessment: report of an ECETOC workshop. Regul. Toxicol. Pharmacol. 91 (Suppl. 1), S3–S13.

Chang, X., Kleinstreuer, N., Ceger, P., Hsieh, J.-H., Allen, D., Casey, W., 2015. Application of reverse dosimetry to compare *in vitro* and *in vivo* estrogen receptor activity. Appl. In Vitro Toxicol. 1 (1), 33–44.

Chapman, K.L., Holzgrefe, H., Black, L.E., Brown, M., Chellman, G., Copeman, C., Couch, J., Creton, S., Gehen, S., Hoberman, A., Kinter, L.B., Madden, S., Mattis, C., Stemple, H.A., Wilson, S., 2013. Pharmaceutical toxicology: designing studies to reduce animal use, while maximizing human translation. Regul. Toxicol. Pharmacol. 66 (1), 88–103.

Clewell 3rd, H.J., Andersen, M.E., Blaauboer, B.J., 2008. On the incorporation of chemical-specific information in risk assessment. Toxicol. Lett. 180 (2), 100–109.

Commission, 2013. Commission Regulation No 283/2013 of 1 March 2013 setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. O.J. L 93, 1–84 3 Apr 2013.

Cramer, G.M., Ford, R.A., Hall, R.L., 1978. Estimation of toxic hazard - a decision tree approach. Food Chem. Toxicol. 16, 255–276. cf. also. https://eurl-ecvam.jrc.ec.europa.eu/laboratories-research/predictive_toxicology/background/TTC.

de Boer, J., Fritsche, E., Schoeters, G., Kimber, I., 2015. The European Long-range Research Initiative (LRI): a decade of contributions to human health protection, exposure modelling and environmental integrity. Toxicology 337, 83–90.

de Miranda Silva, C., Rocha, A., Tozatto, E., da Silva, L.M., Donadi, E.A., Dalla Costa, T., Lanchote, V.L., Schmidt, S., Bulitta, J.B., 2017. Development of an enantioselective and biomarker-informed translational population pharmacokinetic/pharmacodynamic model for etodolac. AAPS J. 19 (6), 1814–1825.

DeWoskin, R.S., Sweeney, L.M., Teeguarden, J.G., Sams, R. 2nd, Vandenberg, J., 2013. Comparison of PBTK model and biomarker based estimates of the internal dosimetry of acrylamide. Food Chem. Toxicol. 58, 506–521.

Donato, M.T., Castell, J.V., 2003. Strategies and molecular probes to investigate the role of cytochrome P450 in drug metabolism: focus on *in vitro* studies. Clin. Pharmacokinet. 42 (2), 153–178.

Dorne, J.L.C.M., 2001. Human Variability in Kinetics for the Major Metabolic Pathways-application to Chemical Risk Assessment. PhD thesis. University of Southampton, UK 300 pp.

Dorne, J.L., Walton, K., Renwick, A.G., 2005. Human variability in xenobiotic metabolism and pathway-related uncertainty factors for chemical risk assessment: a review. Food Chem. Toxicol. 43 (2), 203–216.

Dorne, J.L., Richardson, J., Kass, G., Georgiadis, N., Monguidi, M., Pasinato, L., Cappe, S., Verhagen, H., Robinson, T., 2017. Editorial: OpenFoodTox: EFSA's open source toxicological database on chemical hazards in food and feed. EFSA J 15 (1), e15011.

ECETOC, 2012. European Centre for Ecotoxicology and Toxicology of Chemicals Technical Report No. 116. Category Approaches, Read-across, (Q)SAR. Brussels, Belgium, Nov 2012.

ECETOC, 2017. European Centre for Ecotoxicology and Toxicology of Chemicals. Seven Steps for the Identification of Endocrine Disrupting Properties (ECETOC 7SI-ed). ECETOC TR 130, Belgium, Mar 2017.

ECHA, 2013. European Chemicals Agency. Grouping of Substances and Read-across Approach. Part 1: Introductory Note. ECHA-13-R-02-EN, April 2013.

ECHA, 2016. European Chemicals Agency. Report on the Operation of REACH and CLP 2016. ECHA-16-R-08-EN, May 2016.

ECHA, 2017a. European Chemicals Agency. Read-across Assessment Framework (RAAF). ECHA-17-R-01-EN, Mar 2017.

ECHA, 2017b. European Chemicals Agency. Report. Current Status of Regulatory Applicability under the REACH, CLP and Biocidal Products Regulations. ECHA-17/R/24/EN, Nov 2017.

EFSA, 2014a. European Food Safety Authority. Scientific Report. Modern methodologies and tools for human hazard assess of chemical. EFSA J 12 (4), 3638.

EFSA, 2014b. European Food Safety Authority. Technical Report. Outcome of the Public Consultation on the Scientific Opinion on the Identification of Pesticides to Be Included in Cumulative Assessment Groups (CAGs) on the Basis of Their Toxicological Profile. EFSA supporting publication 2014:EN-538, 53 pp.

EFSA Scientific Committee, 2012. Scientific Opinion on Exploring options for providing advice about possible human health risks based on the concept of Threshold of Toxicological Concern (TTC). EFSA J 10 (7), 2750.

EFSA Scientific Committee, 2017. European Food Safety Authority Scientific Committee. Guidance on the use of the weight of evidence approach in scientific assessments. EFSA J 15 (8), 4971.

EMA, 1994. European Medicines Agency. ICH Topic S5 (R2/3)) Detection of toxicity to reproduction for medicinal products including toxicity to male fertility. CPMP/ICH/386/95, Mar 1994, available at. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002809.pdf.

EMA, 1995a. European Medicines Agency. ICH Topic S3A Toxicokinetics: a guidance for assessing systemic exposure in toxicology studies. CPMP/ICH/384/95, Jun 1995, available at. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002770.pdf.

EMA, 1995b. European Medicines agency. ICH topic S3B pharmacokinetics: repeated-dose tissue distribution studies. CPMP/ICH/385/95, Jun 1995, available at. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002771.pdf.

EMA, 2008. ICH harmonised tripartite guideline: dose selection for carcinogenicity studies of pharmaceuticals S1C (R2). CPMP/ICH/383/95, Oct 2008, available at. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002768.pdf.

EMA, 2009. European Medicines Agency. ICH guideline M3 (R2) on non-clinical safety studies for the conduct of human clinical trials and marketing authorisation for pharmaceuticals. EMA(CPMP/IC/286/1995, Dec 2009, available at. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002720.pdf.

EP and Council, 2006. Regulation (EC) No 1907/2006 of the european parliament and of the Council of 18 december 2006 concerning the registration, evaluation, authorisation and restriction of chemicals (REACH), establishing a european chemicals agency, amending directive 1999/45/EC and repealing Council regulation (EEC) No 793/93 and commission regulation (EC) No 1488/94 as well as Council directive 76/769/EEC and commission directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC. O.J. L 396/1 30 Dec 2006.

EP and Council, 2008. Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006. O.J. L 353, 1–1355 31 Dec 2008.

EP and Council, 2009a. Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. O.J. L 309, 1–50 24 Nov 2009.

EP and Council, 2009b. Regulation (EC) No. 1223/2009 of the european parliament and of the Council of 30 november 2009 on cosmetic products. O.J. L 342/59, 22 Dec 2009.

EP and Council, 2010. Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. O.J. L 276/33, 20 Oct 2010.

EP and Council, 2012. Regulation (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products. O.J. L 167/1, 27 Jun 2012.

Fisher, C., Hatley, O., Gardner, I., Jamei, M., 2016. A multi-compartment liver model for the prediction of toxicokinetics. Toxicol. Lett. 258 (Suppl. l) S121.

Gajewska, M., Paini, A., Sala Benito, J.V., Burton, J., Worth, A., Urani, C., Briesen, H., Schramm, K.-W., 2015. *In vitro*-to-*in vivo* correlation of the skin penetration, liver clearance and hepatotoxicity of caffeine. Food Chem. Toxicol. 75, 39–49.

Gao, H., Jacobs, A., White, R.E., Booth, B.P., Obach, R.S., 2013. Meeting report: metabolites in safety testing (MIST) symposium-safety assessment of human metabolites: what's REALLY necessary to ascertain exposure coverage in safety tests? AAPS J. 15, 970–973.

Gerstel, D., Jacques-Jamin, C., Schepky, A., Cubberley, R., Eilstein, J., Grégoire, S., Hewitt, N., Klaric, M., Rothe, H., Duplan, H., 2016. Comparison of protocols for measuring cosmetic ingredient distribution in human and pig skin. Toxicol. Vitro 34, 153–160.

Gocht, T., Berggren, E., Ahr, H.J., Cotgreave, I., Cronin, M.T., Daston, G., Hardy, B., Heinzle, E., Hescheler, J., Knight, D.J., Mahony, C., Peschanski, M., Schwarz, M., Thomas, R.S., Verfaillie, C., White, A., Whelan, M., 2015. The SEURAT-1 approach towards animal free human safety assessment. ALTEX 32 (1), 9–24.

Goldman, M., Seigneuret, N., Eichler, H.G., 2015. The Innovative Medicines Initiative: an engine for regulatory science. Nat. Rev. Drug Discov. 14 (1), 1–2.

Hand, L.H., Richardson, K., Hadfield, S.T., Whalley, S., Rawlinson, P., Booth, E.D., 2017.

Use of read-across to simplify the toxicological assessment of a complex mixture of lysimeter leachate metabolites on the basis of chemical similarity and ADME behavior. Regul. Toxicol. Pharmacol. 83, 109–116.
Horiuchi, K., Ohnishi, S., Matsuzaki, T., Funaki, S., Watanabe, A., Mizutare, T., Matsumoto, S., Nezasa, K.I., Hasegawa, H., 2018. Improved human pharmacokinetic prediction of hepatically metabolized drugs with species-specific systemic clearance. J. Pharmacol. Sci Epub ahead of print, pii: S0022-3549(17)30909-7.
ICH, 2017. International Conference on Harmonisation. Questions and Answers to ICHS3A: Note for Guidance on Toxicokinetics: the Assessment of Systemic Exposure in Toxicity Studies Focus on Microsampling. S3A Q&As.
Jacques-Jamin, C., Duplan, H., Rothe, H., Vaillant, O., Eilstein, J., Grégoire, S., Cubberley, R., Lange, D., Ellison, C., Klaric, M., Hewitt, N., Schepky, A., 2017. Comparison of the skin penetration of 3 metabolically stable chemicals using fresh and frozen human skin. Skin Pharmacol. Physiol. 30 (5), 234–245.
Klaasen, C.D., Cassarett, L.J., Doull, J. (Eds.), 2013. Casarett and Doull's Toxicology – the Basic Science of Poisons, eighth ed. Mcgraw Hill Education, New York, USA.
Klein, S., Maggioni, S., Bucher, J., Mueller, D., Niklas, J., Shevchenko, V., Mauch, K., Heinzle, E., Noor, F., 2016. *In silico* modeling for the prediction of dose and pathway-related adverse effects in humans from *in vitro* repeated-dose studies. Toxicol. Sci. 149, 55–66.
Kroes, R., Galli, C., Munro, I., Schilter, B., Tran, L., Walker, R., Würtzen, G., 2000. Threshold of toxicological concern for chemical substances present in the diet: a practical tool for assessing the need for toxicity testing. Food Chem. Toxicol. 38 (2–3), 255–312.
Laufersweiler, M.C., Gadagbui, B., Baskerville-Abraham, I.M., Maier, A., Willis, A., Scialli, A.R., Carr, G.J., Felter, S.P., Blackburn, K., Daston, G., 2012. Correlation of chemical structure with reproductive and developmental toxicity as it relates to the use of the threshold of toxicological concern. Regul. Toxicol. Pharmacol. 62 (1), 160–182.
Loizou, G., Hogg, A., 2011. MEGen: a physiologically based pharmacokinetic model generator. Front. Pharmacol. 2, 56.
Long, J.C., Cunningham, F.C., Braithwaite, J., 2013. Bridges, brokers and boundary spanners in collaborative networks: a systematic review. BMC Health Serv. Res. 13, 158.
Lu, J., Goldsmith, M.R., Grulke, C.M., Chang, D.T., Brooks, R.D., Leonard, J.A., Phillips, M.B., Hypes, E.D., Fair, M.J., Tornero-Velez, R., Johnson, J., Dary, C.C., Tan, Y.M., 2016. Developing a physiologically-based pharmacokinetic model knowledgebase in support of provisional model construction. PLoS Comput. Biol. 12 (2) e1004495.
Maertens, A., Hubesch, B., Rovida, C., 2016. Two good read-across practice workshops. Making it work for you!. ALTEX 33, 324–326.
Martínez, M.A., Rovira, J., Sharma, R.P., Nadal, M., Schuhmacher, M., Kumar, V., 2017. Prenatal exposure estimation of BPA and DEHP using integrated external and internal dosimetry: a case study. Environ. Res. 158, 566–575.
Meek, M.E., Boobis, A., Cote, I., Dellarco, V., Fotakis, G., Munn, S., Seed, J., Vickers, C., 2014a. New developments in the evolution and application of the WHO/IPCS framework on mode of action/species concordance analysis. J. Appl. Toxicol. 34, 1–18.
Meek, M.E., Palermo, C.M., Bachman, A.N., North, C.M., Jeffrey Lewis, R., 2014b. Mode of action human relevance (species concordance) framework: evolution of the Bradford Hill considerations and comparative analysis of weight of evidence. J. Appl. Toxicol. 34, 595–606.
Munro, I.C., Ford, R.A., Kennepohl, E., Sprenger, J.G., 1996. Correlation of structural class with no-observed-effect levels: a proposal for establishing a threshold of concern. Food Chem. Toxicol. 34, 829–867.
Noorlander, C., Zeilmaker, M., van Eijkeren, J., Bourgeois, F., Beffers, R., Brandon, E., Bessems, J., 2008. Data Collection on Kinetic Parameters of Substances. Pilot Phase – a Methodological Report. CCR.IHCP .C432921.XO. National Institute for Public Health and the Environment (RIVM), The Netherlands available at. https://eurl-ecvam.jrc.ec.europa.eu/validation-regulatory-acceptance/docs-toxicokinetics/Methodological%20report.pdf.
OECD, 2004a. Organisation for Economic Co-operation and Development. Series on Testing and Assessment No. 28. Guidance Document for the Conduct of Skin Absorption Studies. ENV/JM/MONO(2004)2. OECD, Paris, France 05 Mar 2004.
OECD, 2004b. Organisation for Economic Co-operation and Development Guideline for the Testing of Chemicals 428. Skin Absorption: *in Vitro* Method. OECD, Paris, France 13 Apr 2004.
OECD, 2010. Organisation for Economic Co-operation and Development. Guideline for the Testing of Chemicals 417. Toxicokinetics. OECD, Paris, France 23 Jul 2010.
OECD, 2011. Organisation for Economic Co-operation and Development. Series on Testing and Assessment No. 156. Guidance Notes on Dermal Absorption. ENV/JM/MONO(2011)36. OECD, Paris, France 18 Aug 2011.
OECD, 2013. Organisation for Economic Co-operation and Development. Series on Testing and Assessment No. 184. Revised Guidance Document on Developing and Assessing Adverse Outcome Pathways, second ed. ENV/JM/MONO(2013)6, Paris, France, 27 Jul 2017.
OECD, 2014. Organisation for Economic Co-operation and Development. Series on Testing and Assessment No. 194. Guidance on Grouping of Chemicals. ENV/JM/MONO(2014)4. second ed. OECD, Paris, France 14 Apr 2014.
OECD, 2015. Organisation for Economic Co-operation and Development. Series on Testing and Assessment No. 229. Fundamental and Guiding Principles for (Q)SAR Analysis of Chemical Carcinogenicity with Mechanistic Considerations. ENV/JM/MONO(2015)46. OECD, Paris, France 03 Dec 2015.
OECD, 2016a. Organisation for Economic Co-operation and Development. Series on Testing and Assessment No. 250. Report on Considerations from Case Studies on Integrated Approaches for Testing and Assessment (IATA). First Review Cycle (2015). Case Studies on Grouping Methods as a Part of IATA. ENV/JM/MONO(2016)48. OECD, Paris, France 12 Sep 2016.
OECD, 2016b. Organisation for Economic Co-operation and Development. Series on Testing and Assessment No. 260. Guidance Document for the Use of Adverse Outcome Pathways in Developing Integrated Approaches to Testing and Assessment (IATA) ENV/JM/MONO(2016)67. OECD, Paris, France 19 Dec 2016.
OECD, 2017a. Organisation for Economic Co-operation and Development. Series on Testing and Assessment No. 270. Report on Considerations from Case Studies on Integrated Approaches for Testing and Assessment (IATA). Second Review Cycle (2016). ENV/JM/MONO(2017)22. OECD, Paris, France 05 Oct 2017.
OECD, 2017b. Organisation for Economic Co-operation and Development. Series on Testing and Assessment No. 233. Users' Handbook Supplement to the Guidance Document for Developing and Assessing Adverse Outcome Pathways. ENV/JM/MONO(2016)12. OECD, Paris, France 27 Jan 2017.
Paini, A., Joossens, E., Bessems, J., Desalegn, A., Dorne, J.L., Gosling, J.P., Heringa, M.B., Klaric, M., Kramer, N., Loizou, G., Louisse, J., Lumen, A., Madden, J.C., Patterson, E.A., Proença, S., Punt, A., Setzer, R.W., Suciu, N., Troutman, J., Yoon, M., Worth, A., Tan, Y.M., 2017a. EURL ECVAM Workshop on New Generation of Physiologically-based Kinetic Models in Risk Assessment. EUR 28794 EN. Publications Office of the European Union, Luxembourg. https://doi.org/10.2760/035371. ISBN 978-92-79-73849-4.
Paini, A., Leonard, J.A., Kliment, T., Tan, Y.M., Worth, A., 2017b. Investigating the state of physiologically based kinetic modelling practices and challenges associated with gaining regulatory acceptance of model applications. Regul. Toxicol. Pharmacol. 90, 104–115.
Partosch, F., Mielke, H., Stahlmann, R., Kleuser, B., Barlow, S., Gundert-Remy, U., 2015. Internal threshold of toxicological concern values: enabling route-to-route extrapolation. Arch. Toxicol. 89 (6), 941–948.
Patlewicz, G., Ball, N., Boogaard, P.J., Becker, R.A., Hubesch, B., 2015. Building scientific confidence in the development and evaluation of read-across. Regul. Toxicol. Pharmacol. 72 (1), 117–133.
Pearce, R.G., Setzer, R.W., Strope, C.L., Sipes, N.S., Wambaugh, J.F., 2017. httk: R package for high-throughput toxicokinetics. J. Stat. Software 79 (4), 1–26. https://doi.org/10.18637/jss.v079.i04.
Press, B., 2011. Optimization of the Caco-2 permeability assay to screen drug compounds for intestinal absorption and efflux. Meth. Mol. Biol. 763, 139–154.
Punt, A., Schiffelers, M.J., Jean Horbach, G., van de Sandt, J.J., Groothuis, G.M., Rietjens, I.M., Blaauboer, B.J., 2011. Evaluation of research activities and research needs to increase the impact and applicability of alternative testing strategies in risk assessment practice. Regul. Toxicol. Pharmacol. 61 (1), 105–114.
R Core Team, 2013. A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria available at. https://www.r-project.org.
Ramirez, T., Beken, S., Chlebus, M., Ellis, G., Griesinger, C., De Jonghe, S., Manou, I., Mehling, A., Reisinger, K., Rossi, L.H., van Benthem, J., van der Laan, J.W., Weissenhorn, R., Sauer, U.G., 2015. Knowledge sharing to facilitate regulatory decision-making in regard to alternatives to animal testing: report of an EPAA workshop. Regul. Toxicol. Pharmacol. 73, 210–226.
Renwick, A.G., Lazarus, N.R., 1998. Human variability and noncancer risk assessment - an analysis of the default uncertainty factor. Regul. Toxicol. Pharmacol. 27 (1 Pt 1), 3–20.
Rothe, H., Obringer, C., Manwaring, J., Avci, C., Wargniez, W., Eilstein, J., Hewitt, N., Cubberley, R., Duplan, H., Lange, D., Jacques-Jamin, C., Klaric, M., Schepky, A., Grégoire, S., 2017a. Comparison of protocols measuring diffusion and partition coefficients in the stratum corneum. J. Appl. Toxicol. 37 (7), 806–816.
Rothe, H., Grégoire, S., Obringer, C., Manwaring, J., Eilstein, J., Lange, D., Cubberley, R., Duplan, H., Jacques-Jamin, C., Ellison, C., Klaric, M., Hewitt, N., Schepsky, A., 2017b. Skin penetration *in silico* modelling - improvement by using *in vitro* data. Toxicol. Lett. 280 (Suppl. 1), S45.
Russell, W.M.S., Burch, R.L., 1959. The Principles of Humane Experimental Technique. London, UK. Methuen. Reprinted by UFAW, 1992: 8 Hamilton Close, South Mimms, Potters Bar, Herts EN6 3QD England, 238 pp (1959).
Saghir, S.A., Bartels, M.J., Rick, D.L., McCoy, A.T., Rasoulpour, R.J., Ellis-Hutchings, R.G., Sue Marty, M., Terry, C., Bailey, J.P., Billington, R., Bus, J.S., 2012. Assessment of diurnal systemic dose of agrochemicals in regulatory toxicity testing - an integrated approach without additional animal use. Regul. Toxicol. Pharmacol. 63 (2), 321–332.
SCCS, 2015. Scientific Committee on Consumer Safety. The SCCS Notes of Guidance for the Testing of Cosmetic Substances and Their Safety Evaluation. 9th Revision Adopted at the 11th Plenary Meeting of the SCCS on 29 Sep 2015. SCCS/1564/15.
Sharma, R., Strelevitz, T.J., Gao, H., Clark, A.J., Schildknegt, K., Obach, R.S., Ripp, S.L., Spracklin, D.K., Tremaine, L.M., Vaz, A.D., 2012. Deuterium isotope effects on drug pharmacokinetics. I. System-dependent effects of specific deuteration with aldehyde oxidase cleared drugs. Drug Metab. Dispos. 40 (3), 625–634.
Sharma, R.P., Schuhmacher, M., Kumar, V., 2017a. Review on crosstalk and common mechanisms of endocrine disruptors: scaffolding to improve PBPK/PD model of EDC mixture. Environ. Int. 99, 1–14.
Sharma, R.P., Schuhmacher, M., Kumar, V., 2017b. Developing integrated PBPK/PD coupled mechanistic pathway model (miRNA-BDNF): an approach towards system toxicology. Toxicol. Lett. 280, 79–91.
Sharma, R.P., Schuhmacher, M., Kumar, V., 2018. The development of a pregnancy PBPK Model for Bisphenol A and its evaluation with the available biomonitoring data. Sci. Total Environ. 624, 55–68.
Shebley, M., Sandhu, P., Emami Riedmaier, A., Jamei, M., Narayanan, R., Patel, A., Peters, S.A., Reddy, V.P., Zheng, M., de Zwart, L., Beneton, M., Bouzom, F., Chen, J., Chen, Y., Cleary, Y., Collins, C., Dickinson, G.L., Djebli, N., Einolf, H.J., Gardner, I., Huth, F., Kazmi, F., Khalil, F., Lin, J., Odinecs, A., Patel, C., Rong, H., Schuck, E., Sharma, P., Wu, S.P., Xu, Y., Yamazaki, S., Yoshida, K., Rowland, M., 2018. Physiologically based pharmacokinetic model qualification and reporting procedures for regulatory submissions: a consortium perspective. Clin. Pharmacol. Ther. 104 (1),

88–110.
Schultz, T.W., Amcoff, P., Berggren, E., Gautier, F., Klaric, M., Knight, D.J., Mahony, C., Schwarz, M., White, A., Cronin, M.T., 2015. A strategy for structuring and reporting a read-across prediction of toxicity. Regul. Toxicol. Pharmacol. 72 (3), 586–601.
Schultz, T.W., Cronin, M.T.D., 2017. Lessons learned from read-across case studies for repeated-dose toxicity. Regul. Toxicol. Pharmacol. 88, 185–191.
Shen, J., Kromidas, L., Schultz, T., Bhatia, S., 2014. An *in silico* skin absorption model for fragrance materials. Food Chem. Toxicol. 74, 164–176.
Terry, C., Hays, S., McCoy, A.T., McFadden, L.G., Aggarwal, M., Rasoulpour, R.J., Juberg, D.R., 2016. Implementing a framework for integrating toxicokinetics into human health risk assessment for agrochemicals. Regul. Toxicol. Pharmacol. 75, 89–104.
Toropov, A.A., Toropova, A.P., Marzo, M., Dorne, J.L., Georgiadis, N., Benfenati, E., 2017. QSAR models for predicting acute toxicity of pesticides in rainbow trout using the CORAL software and EFSA's OpenFoodTox database. Environ. Toxicol. Pharmacol. 53, 158–163.
Toropova, A.P., Toropov, A.A., Marzo, M., Escher, S.E., Dorne, J.L., Georgiadis, N., Benfenati, E., 2018. The application of new HARD-descriptor available from the CORAL software to building up NOAEL models. Food Chem. Toxicol. 112, 544–550.
Wambaugh, J.F., Hughes, M.F., Ring, C.L., MacMillan, D.K., Ford, J., Fennell, T.R., Black, S.R., Snyder, R.W., Sipes, N.S., Wetmore, B., Westerhout, J., Setzer, R.W., Pearce, R., Simmons, J.E., Thomas, R.S., 2018. Evaluating *in vitro-in vivo* extrapolation of toxicokinetics. Toxicol. Sci. https://doi.org/10.1093/toxsci/kfy020. Epub ahead of print 27 Jan 2018.
Weed, D.L., 2005. Weight of evidence: a review of concept and methods. Risk Anal. 25, 1545–1557.
Whalley, P.M., Bartels, M., Bentley, K.S., Corvaro, M., Funk, D., Himmelstein, M.W., Neumann, B., Strupp, C., Zhang, F., Mehta, J., 2017. An *in vitro* approach for comparative interspecies metabolism of agrochemicals. Regul. Toxicol. Pharmacol. 88, 322–327.
WHO, 2009. World Health Organization Environmental Health Criteria 240: Principles and Methods for the Risk Assessment of Chemicals in Food. WHO, Geneva, CH. http://www.who.int/foodsafety/publications/chemical-food/en/.
WHO-UNEP, 2012. In: Bergman, A., Heindel, J.J., Jobling, S., Kidd, K.A., Zoeller, R.T. (Eds.), World Health Organization - United Nations Environment Programme. State of the Science of Endocrine Disrupting Chemicals. Summary for Decision-makers, . http://www.who.int/ceh/publications/endocrine/en/index.html, Accessed date: 9 January 2018.
Wood, F.L., Houston, J.B., Hallifax, D., 2017. Clearance prediction methodology needs fundamental improvement: trends common to rat and human hepatocytes/microsomes and implications for experimental methodology. Drug Metab. Dispos. 45 (11), 1178–1188.