**Title:** Next generation physiologically based kinetic (NG-PBK) models in support of regulatory decision making

**Authors:** Paini A1\*, Leonard JA2, Joossens E1, Bessems JGM1, 18, Desalegn A1, Dorne JL3, Gosling JP4, Heringa MB5, Klaric M6, Kliment T1, Kramer NI7, Loizou G8, Louisse J9, 13, Lumen A10, Madden JC11, Patterson EA12, Proença S1,7, Punt A13, Setzer RW14, Suciu N15, Troutman J16, Yoon M17, 19, Worth A1, Tan YM14.

\*Corresponding Author: Alicia.paini@ec.europa.eu

Affiliations

1 European Commission Joint Research Centre, Ispra, Italy.

2 Oak Ridge Institute for Science and Education, 100 ORAU Way, Oak Ridge, TN 37830, USA.

3 European Food Safety Authority, 1a, Via Carlo Magno, 1A, 43126 Parma PR, Italy.

4 School of Mathematics, University of Leeds, Leeds, U.K.

5 RIVM - The National Institute for Public Health and the Environment, Bilthoven, The Netherlands.

6 Cosmetics Europe, Brussels, Belgium

7 Institute for Risk Assessment Sciences, Utrecht University, P.O. Box 80177, 3508TD Utrecht, The Netherlands.

8 Health and Safety Laboratory, Buxton, UK

9 Division of Toxicology, Wageningen University, Tuinlaan 5, 6703 HE Wageningen, The Netherlands.

10 Division of Biochemical Toxicology, National Center for Toxicological Research, US Food and Drug Administration, Jefferson, AR 72079, USA.

11 School of Pharmacy and Bimolecular Sciences, Liverpool John Moores University, Byrom Street, Liverpool L3 3AF, UK.

12 School of Engineering, University of Liverpool, Liverpool, L69 3GH, UK.

13.RIKILT Wageningen University and Research, Akkermaalsbos 2, 6708 WB, Wageningen, The Netherlands.

14 U.S. Environmental Protection Agency, National Exposure Research Laboratory, 109 TW Alexander Drive, Research Triangle Park, NC 27709, USA

15.DiSTAS, Università Cattolica del Sacro Cuore, Piacenza, Italy.

16.Central Product Safety, The Procter & Gamble Company, Cincinnati, OH, USA.

17 ScitoVation, 6 Davis Drive, PO Box 110566, Research Triangle Park, NC 27709, USA

18 Flemish Institute for Technological Research (VITO), Mol, Belgium

19ToxStrategies, Research Triangle Park Office, 1249 Kildaire Farm Road 134, Cary, NC 27511, USA

**Highlights**

PBK models have helped to facilitate quantitative *in vitro* to *in vivo* extrapolation.

PBK modelling has played a significant role in reducing animal testing.

It is critical to assess the validity and credibility of PBK models built using non-animal data.

There is a need to train regulators to better understand PBK modelling.

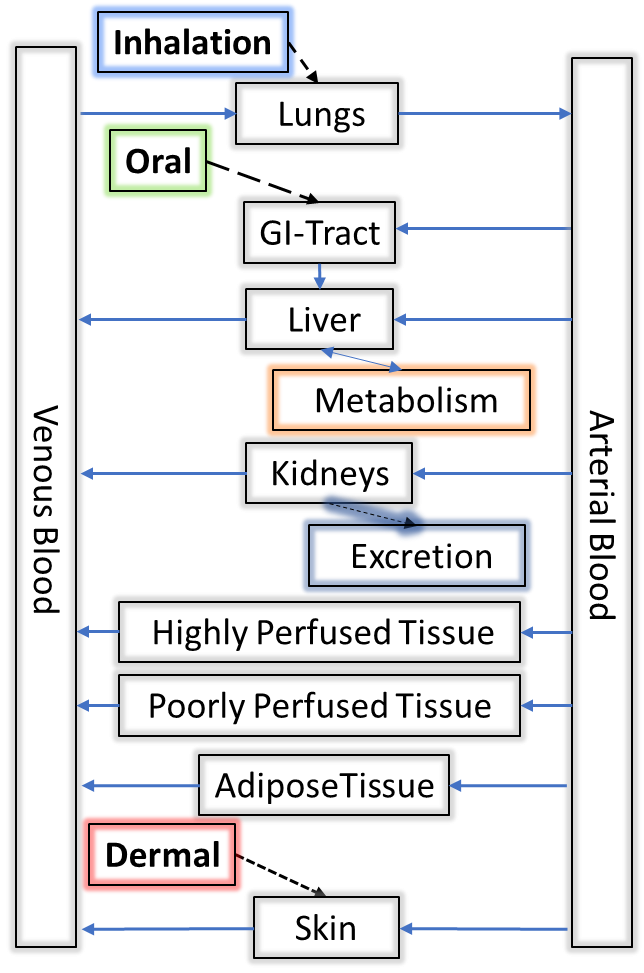
**Abstract**

Currently, the field of toxicology and chemical risk assessment seek to reduce, and eventually replace, the use of animals for the prediction of toxicity in humans. In this context, data from next-generation methodology (NGM) and physiologically based kinetic (PBK) modelling have played a significant role in reducing animal testing, by providing a platform capable of incorporating NGM and *in vitro* human data to facilitate the development of *in vitro* to *in vivo* extrapolation models. In the present article, we discuss the challenges in: 1) applying PBK modelling to support regulatory decision making under the toxicology and risk-assessment paradigm shift; 2) constructing PBK models without *in vivo* animal kinetic data, while relying solely on *in vitro* or *in silico* methods for model parameterization; and 3) assessing the validity and credibility of PBK models built largely using non-animal data. The strengths, uncertainties, and limitations of PBK models developed using NGM data are discussed in an effort to establish a higher degree of confidence in the application of such models in a regulatory context. The discussions provided in this article summarize the outcome of an expert workshop hosted by the European Commission Joint Research Centre (EC-JRC) - European Union Reference Laboratory for Alternatives to Animal Testing (EURL-ECVAM), on “Physiologically-Based Kinetic modelling in risk assessment – reaching a whole new level in regulatory decision-making” held in Ispra, Italy, in November 2016, along with recently reported activities occurring within the PBK model field and the results from an international survey conducted in 2017. The discussions presented herein will aid the broader scientific and regulatory communities to better understand the applications of PBK modelling.

**Keywords**: Physiologically Based Pharmacokinetic models; PBPK; PBTK; Toxicokinetics; NAMs

**Introduction**

Modelling and simulation based approaches are gradually gaining interest as critical tools for safety and risk assessment of a variety of compounds including drugs, chemicals, consumer products, and food ingredients. These modelling approaches are recognized for the crucial role they play in, for example, predicting the biokinetics of drugs and chemicals in the organism without the need to conduct *in vivo* experiments. For more than 40 years, physiologically-based kinetic (PBK) models have been used to simulate biokinetics (Andersen and Krishnan, 2010; Mumtaz et al., 2012; Krishnan and Peyret, 2009; Bois et al., 2017). In PBK models, the body is represented as a series of interconnected compartments linked via blood flow, as depicted in the schematic figure 1a, to simulate concentration-time curves in target organs or their surrogates, such as in blood (figure 1b). PBK models use differential equations to describe the absorption, distribution, metabolism, and elimination (ADME) processes that govern the fate and transport of the chemical among these interconnected compartments. Proper use of PBK models helps to reduce uncertainties and to identify data gaps inherent in hazard characterization approaches that rely upon default extrapolation factors (e.g., a multiplication factor of 10 for inter-species extrapolation) to derive health-based guidance values from animal toxicity studies. PBK models provide a sound scientific basis to extrapolate across species, routes of exposure, and exposure scenarios, based on physiology and (physico)-chemical properties (Loizou et al., 2008; Bessems et al., 2014). As PBK models can be developed for specific individuals within the human population, they provide a means for quantifying inter-individual differences in kinetics, allowing for the determination of extrapolation factors across age groups or across populations of varying susceptibilities. With this information, safe chemical intake levels can be derived for individuals and populations. Most recently, PBK models have helped to facilitate quantitative *in vitro* to *in vivo* extrapolation (QIVIVE) approaches (Yoon et al., 2012, 2014, 2015; Wetmore et al., 2015; Louisse et al., 2017), enabling the use of *in vitro* toxicity data for the setting of safe intake levels. QIVIVE is an essential process in linking an *in vitro* measured biological (adverse) readout to a potential *in vivo* outcome (Groothuis et al., 2015). QIVIVE provides a means of considering exposure and dosimetry, and enables the use of *in vitro* toxicity data for risk-based assessments beyond hazard-based assessments (Bell et al., 2018). Once an *in vitro* concentration-response has been generated, the benchmark dose approach can be applied to the predicted dose – response data, to obtain an *in vitro-*based point of departure (PoD) or Reference Point (RfP) (Louisse et al., 2015; 2017).

**a**

**Figure 1**. (a) Schematic representation of a physiologically based kinetic (PBK) model, (b) with an example of a typical PBK model-output (time-dependent chemical concentration in blood).

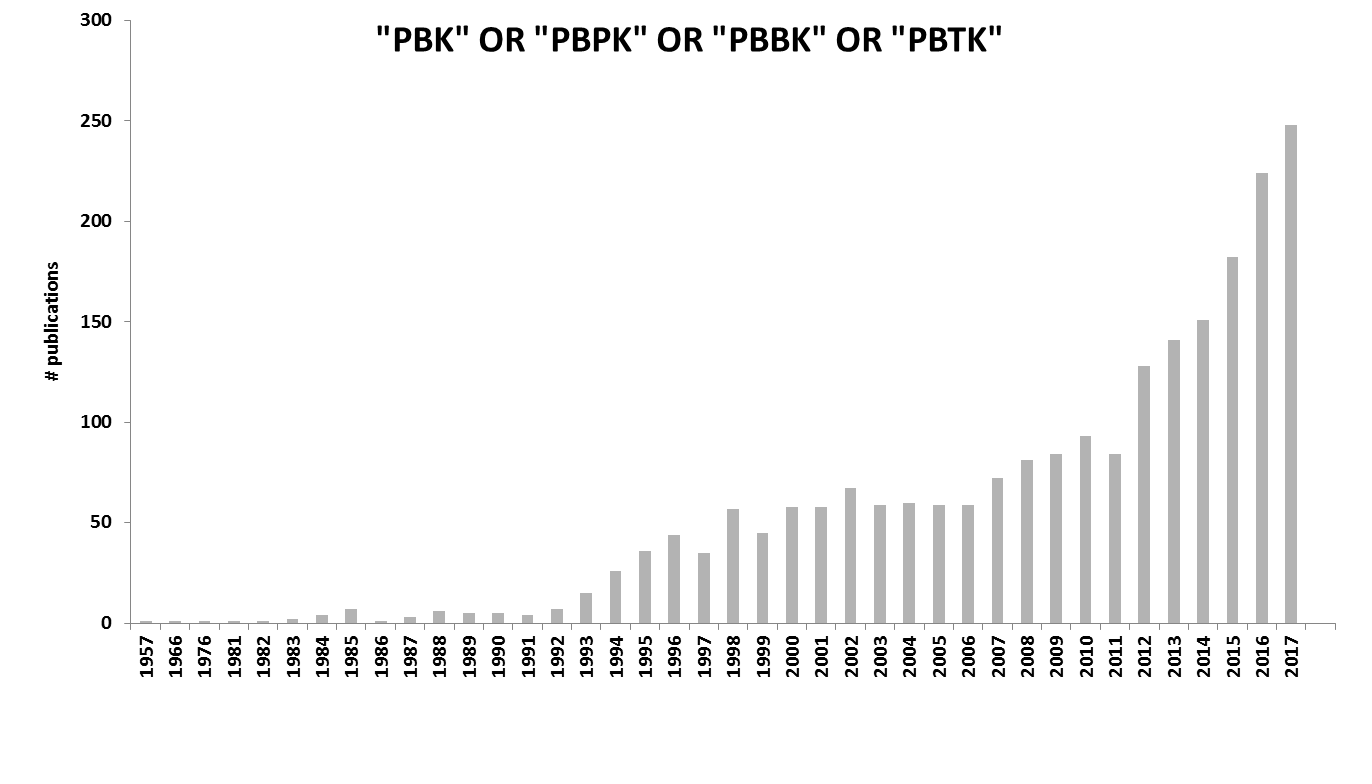
**Nomenclature**

“Physiologically based pharmacokinetic” (PBPK) model is the most widely used term and was developed by the pharmaceutical field to simulate the kinetics of drugs. Despite the popular use of the term, “PBPK” is not entirely correct in the context of general chemical risk assessment. Another term preferred in the European Union (EU) and related to chemical risk assessment is “PBTK”, with TK standing for “toxicokinetics”. However, this term is not entirely appropriate either (Clewell et al., 2008). Rather, a more general nomenclature, such as physiologically based biokinetic (PBBK) or the aforementioned PBK, might be seen as more appropriate. Regardless of the terminology used, PBK, PBPK, PBBK and PBTK can all be considered synonyms, and so throughout this document we will consistently use the more general terms of PBK model or PBK modelling. It is noted that, due to the ever-increasing advancements and next-generation methodologies (NGM) in the field of toxicology, NGM can be used in combination with PBK models to support regulatory decisions for the use of chemical substances. In the present manuscript the term next generation PBK (NG-PBK) model will be used to name these models. This term, NG-PBK, refers to PBK models that are developed without the provision of newly produced (i.e., without animal sacrifice) animal TK data for calibration and validation of those models but rather through NGMs. These models representing the human body should be parameterized and validated using *in vitro*, *in silico*, and human *in vivo* data, when available. This stands also for animal PBK models, where we desire to parameterize and validate using *in vitro*, *in silico*, and historical or monitoring animal data, thus avoiding the need for animal sacrifice.

**Milestones in the history of PBK modelling**

The principles behind PBK modelling were first reported in 1937 by Teorell, in a piece entitled “Kinetics of distribution of substances administered to the body” (Teorell, 1937). Although Teorell’s work was the first attempt to describe the body as a series of equations, the complexity of the mathematics, lack of data, and lack of computing power rendered his concepts incomplete until the 1960s. Between the 1960s and 1970s, several PBK models were developed for pharmaceutical drugs to target cancer diseases (Bischoff and Dedrick 1968; Bischoff et al. 1970). These publications paved the way for more than 2000 articles written on the topic of PB(P/T)K modelling within the last forty years (see figure 2a). Over the past decade, there has been an increase in the development of PBK models for use in a variety of scientific fields, such as pharmacology, forensic sciences, and chemical risk assessment (figure 2b), while such an increase was not registered for toxicology and veterinary medicine. Many risk assessors remain reluctant to apply these models within their work (Paini et al. 2017b, Punt et al., 2017, 2018), as PBK models are not often included in current hazard characterization and risk assessment protocols. In addition, some regulatory agencies may often have limited experience in using PBK models, and the complexity associated with the evaluation of model performance has also contributed to this reluctance.

Over the past 20 years, several workshops have been held to promote the applicability of PBK models in the academic, industrial, and regulatory sectors. For example, a 1995 European Centre for the Validation of Alternative Methods (ECVAM) workshop discussing the use of biokinetic and *in vitro* methods resulted in 15 recommendations that were submitted to support and guide future work in the PBK modelling field (Blaauboer et al., 1996). This workshop was followed by many others to better define the potential role of PBK modelling in science and risk assessment following a Three R (replacement, reduction and refinement) strategy (Bouvier d’Yvoire et al., 2007). In the same year, a workshop to address uncertainty and variability analysis in PBK modeling was held by Barton et al. (2007). Loizou et al. (2008) reported the need for clear descriptions of good modelling practices for: 1) model development; 2) model characterization; 3) model documentation; and 4) model evaluation. A subsequent thematic workshop aimed to critically appraise PBK modelling software platforms and to provide a more detailed state-of-the-art overview of non-animal based PBK parameterisation tools (Bessems et al., 2014). A CEN (European Committee for Standardization) workshop in 2014 strived for achieving the objective of agreement upon the minimum requirements for the amount and type of information to be provided for exposure models, such as PBK models, along with documentation and guidelines for the structure and reporting of such information. The resulting CEN workshop agreement (CWA) was expected to provide a more rigorous means of describing exposure models and to aid users in better understanding them (Ciffroy et al., 2016a; Altenphol et al., 2018). The following year, a workshop assessed the state of knowledge in the application of PBK models in regulatory decision-making, in addition to sharing and discussing best practices in the use of PBK modelling to inform dose selection in specific patient populations (Wagner et al., 2015). In 2017, a workshop organized by the National Centre for the Replacement, Refinement, and Reduction of Animals in Research (NC3Rs) encouraged experts in exposure science to consider the role of PBK models in the extrapolation of external exposure data to internal concentrations to promote the application of non-animal data in efficacy and safety testing (Burden et al., 2017; <https://www.nc3rs.org.uk/applying-exposure-science-increase-utility-non-animal-data-efficacy-and-safety-testing> ). A Lorentz Center workshop entitled “Non-animal Methods for Toxicokinetics: Meeting New Paradigms in Toxicology” was held at the end of 2017 and emphasized the role of PBK models (<https://www.lorentzcenter.nl/lc/web/2017/943/info.php3?wsid=943&venue=Oort>; <https://www.lorentzcenter.nl/lc/web/2017/943/report.pdf>). The first European Partnership for Alternative Approaches to Animal Testing (EPAA) partners’ forum, held at the end of 2017, aimed to provide an overview on toxicokinetics and read across with insight into the role of PBK models (Laroche et al., 2018).

**A.**

**B.**

**Figure 2. A.** Number of papers published per year within the last 60 years. The search was conducted using the online repository PubMed on the 7th of March 2018, with key words string including “PBPK OR PBBK OR PBTK OR PBK”. **B**.The number of papers (figure 2 A) published with key words string including “PBPK OR PBBK OR PBTK OR PBK” were normalized to the following terms: Toxicology; Pharmacology; Chemical Safety OR Risk assessment; Forensic Sciences and Veterinary.

**Framing the problem**

The EURL ECVAM Strategy on Toxicokinetics[[1]](#footnote-1), as published in 2015, outlines opportunities for generating and making better use of TK data. The central feature of the strategy focuses on the use of PBK modelling to integrate data from *in vitro* and *in* *silico* methods for prediction of human whole-body biokinetic behavior, and enables IVIVE to obtain safety guidance values expressed as external doses (Bell et al., 2018). In the past, *in vivo* tissue/blood concentration-time data were a prerequisite for calibrating and evaluating the predictive capability of a PBK model (Bessems, et al., 2014). The common practice was to start with an animal PBK model, calibrating it with animal *in vivo* data, and then re-parameterizing it based on *in vitro* biotransformation measurements or allometric scaling to develop a human PBK model. As the field of risk assessment evolves towards the goal of reducing, and eventually replacing, the use of animals for predicting human toxicity, PBK model development has seen a shift towards increased use of non-animal data for parameterization, along with increased use of the models for IVIVE. Efforts in this area should be directed towards developing standards that will increase the acceptance of *in vitro* methods for characterizing human-relevant ADME properties. To enhance the acceptance of PBK models at an international level, good modelling practice (GMP) is required to guide the use of the NG in developing PBK models. As the first step, to initiate a dialogue on such a topic, the Joint Research Centre (JRC), EURL ECVAM, hosted a workshop on “Physiologically-Based Kinetic modelling in risk assessment – reaching a whole new level in regulatory decision-making” (Ispra, Italy, November 16–17, 2016). The workshop participants discussed challenges in: 1) applying NG-PBK modelling to support regulatory decision making; 2) constructing PBK models for safety assessment without animal *in vivo* data, while relying solely on *in vitro* or *in silico* methods; 3) salient features: Integrating NG-PBK modelling with toxicodynamic endpoints**;** and 4) assessing the validity and credibility of PBK models that rely only upon non-animal data. A portion of this current article summarizes the outcome of the workshop; detailed information on the workshop outcomes can be found in the workshop report (Paini et al., 2017a).

In addition to the EURL ECVAM workshop, an international survey was conducted in 2017 to understand the applications of PBK modelling in the broader scientific and regulatory communities. An aggregate summary, including analysis of the results, has been published (Paini et al., 2017b), while results presented by individual country are available online at <http://apps.klimeto.com/pbk/>. The survey provides insight into the current state of knowledge throughout the PBK modelling and user community, as well as a cursory volunteer contact list of modellers available for peer reviewing models. The main findings of the survey showed that though continuous expansion of the modelling community has allowed PBK models to gain ground for use in various scientific and regulatory risk assessment applications, this remains a slow process, due to a lack of guidance, data, and expertise, which continue to limit widespread acceptance of those models in such applications (Paini et al., 2017b). We also introduce recent reported activities in the field that occurred from the time of the EURL ECVAM 2016 workshop to today, in order to provide an update from the 2016 workshop and additional evidence of hesitancy by public health agencies to apply PBK modelling in their decisions.

**Salient Features: Applying NG-PBK modelling to support regulatory decision making**

As concluded from the 2017 survey (Paini 2017b), training, guidance, and dialogue are three main factors that will enable the successful acceptance of NG-PBK modelling in regulatory decision-making.

1. **Training**

Within the current climate of a willingness to reduce, refine, and replace animal testing through ongoing scientific and technological advancements, it would benefit risk assessors and risk managers to be kept abreast of the development of NGMs. In order to achieve this goal, regulators would require better understanding of a number of novel emerging technologies, in addition to PBK modelling. These include -omics, organ-on-a-chip, high-throughput screening methods, read-across, Adverse Outcome Pathways (AOPs), and IVIVE. Additionally, regulators should be helped to understand how these different approaches are integrated in support of chemical safety assessment. On the other hand, it is not necessary for regulators to have detailed training in all the diverse aspects of PBK models; rather, it may be sufficient to provide tailored training that focuses only on the specific needs of each regulatory sector and, when applicable, cross-sector needs. For example, some risk assessors may need or wish to run a model, and so they would require knowledge of the relevant software and expertise to review and run model codes. Other risk assessors may rely on a model peer review system to check the implementation and reliability of new model codes, and in this case, they might only require training that allows for interpretation of the data and putting modelling predictions into context. Risk assessors can also assemble technical committees that consist of members possessing a range of expertise, to review the model code and interpret model results. The training content/format should also be tailored to achieve maximum effectiveness in understanding the application of models. In addition to the traditional classroom setting, training formats could include webinars, *ad hoc* short courses, and more refined or specialized graduate-level courses. Further, online training could potentially generate a larger audience that would also allow the modelling and user community to continue to expand. Finally, since risk assessors generally place higher confidence in *in vivo* data, there is a need to make courses on alternative *in vitro* and *in silico* methods more accessible, to provide a path forward to acceptance of these NG-PBK model applications in regulatory decision making.

1. **Guidance**

While training is essential, establishing guidance and GMP on PBK model applications intended for regulatory purposes is also critical (Loizou et al., 2008). The GMP should include clear documentation on how to report a model’s scope and purposes, details of model development and evaluation, interpretation of results, and applications of the model in risk assessment (Loizou et al., 2008). It is recommended that the individual(s) or community network(s) responsible for each specific step in the development, evaluation, and application process are clearly identified, to increase transparency and allow end users to request targeted training, if necessary, for a specific topic. The context in which the model is to be used, and thus the scope of the model development or amendment(s), should be amongst the first thing(s) discussed and clearly documented. This is especially important to avoid misuse of a reliable model, such as when results of the simulations are applied for the wrong purpose or when the model is applied outside of its applicability domain.

The WHO-IPCS published, in 2010, a guidance document on characterization and application of PBK models in risk assessment (WHO, 2010). Nevertheless, no comprehensive guidance documentation is currently available for reporting and evaluating NG-PBK models without the use of animal *in vivo* TK data, or for interpreting and applying outputs from these models for human safety assessment. Recently, several efforts have been made to produce such documentation. For example, the Scientific Committee for Consumer Safety (SCCS) considers all available scientific data in their safety evaluation of cosmetic substances, including data generated from PBK modelling. In the most recent Notes of Guidance for the Testing of Cosmetic Ingredients and Their Safety Evaluation (SCCS/1564/15)[[2]](#footnote-2), the SCCS defines the conditions for the use of PBK models submitted for risk assessment purposes. PBK modelling has already been accepted as a tool for risk assessment or for use as supporting information in some of the chemical-specific dossiers evaluated by the SCCS, EFSA, and US-EPA. The SCCS document could act as a starting point or as a template for a new general guidance document. Additionally, the new reporting guidelines from the US Food and Drug Administration (FDA) and European Medicine Agency (EMA) (US FDA, 2016; EMA, 2016), on harmonization of reporting and on qualification of PBK modelling and simulation, can also apply to NG-PBK models. To extended this concept, at the Organisation for Economic Co-operation and Development (OECD), a working group comprised of more than 45 scientists from different areas of scientific expertise are drafting a guidance document for characterizing, validating, and reporting uncertainties in NG-PBK model applications.

**Dialogue and Communication**

While training and guidance are both essential, their maximum benefits cannot be achieved without frequent dialogue between regulators, modellers, and model proponents (chemical registrants). Such frequent dialogue not only allows the proposers to better understand the needs of the regulators, but also allows the regulators to provide modellers with feedback throughout the development, evaluation, and application processes. For example, regulators present at the 2016 ECVAM workshop indicated that they prefer to use the simplest model possible, but would be willing to use more complex models if necessity dictates, as finding sufficient input data is rather challenging. Thus, dialogue can help regulators to convey their needs for specific training and for model features, and help proponents to understand the criteria necessary for regulatory acceptance. In the opposite direction, the regulators can learn what is technically or scientifically essential or impossible. As such dialogue may prove to be time-consuming, establishing a harmonized template for model construction and evaluation will facilitate the process. The template should be flexible enough for any regulatory agency or country, and such a template may also require harmonized and defined "terminologies". To efficiently develop a PBK model to support regulatory risk assessment, modellers and end users (proponents and regulators) need to clearly define their goals of model use and related model requirements at an early stage. For example, if a read- across approach is likely to be applied by the end users, biokinetic data for a pre-determined set of relevant chemicals (target and source chemicals) will constitute important supporting material and should be included in the submission package. In situations where safety assessment is conducted for a new chemical on the market, the following criteria may be used to facilitate regulatory acceptance of a PBK model for this substance: 1) the model should be transparent, with a usable code; 2) model uncertainty should consider biological plausibility, and be clearly described and quantified when possible; 3) uncertainty in exposure scenarios should be characterized, because this uncertainty will propagate to PBK model results; 4) user-friendly platforms should be considered; 5) the model should be fit-for-purpose with no unnecessary additional complexity, and with all required parameters measurable; and 6) the model should consider sufficient coverage of chemical space, to allow for read-across approaches if desired. In cases where the model performance needs to be evaluated using human *in vivo* data, regulators may consider using data that are generated from human trials, such as micro-dosing. It should be emphasized that clinical studies would only be conducted once the safety of the chemical has been established and the clinical investigation represents *de minimus* risk to the subjects.

**Salient Features: Constructing PBK models for safety assessment without animal *in vivo* data**

PBK models are built using three sets of parameters: i) physiological and anatomical parameters, with representative reference parameters taken from the species under study (animal or human); ii) biokinetic / ADME properties, which can be gathered using *in vitro* methods or by fitting the model to a data set; and iii) physico-chemical parameters, which are experimentally derived or obtained using *in silico* approaches such as development of quantitative activity relationship (QSAR) models (Rietjens et al., 2011). A good practice for PBK model construction should include a compound exposure/dosing strategy and design of the study to be simulated (problem formulation). Additionally, exposure descriptions should include route, timeframe of the simulation, and the number of individuals in the simulation necessary for sufficient statistical power analysis. In the case of NG-PBK models, assuming there is no possibility to generate *in vivo* animal data for the model calibration and validation, there are two key pre-requisites to build the model:

* Availability of *in vitro* and *in silico* alternatives to generate ADME properties (including prediction of metabolism) of sufficient quality
* Availability and accessibility of modelling platforms.

1. **Availability of *in vitro* and *in silico* data for ADME properties**

Without *in vivo* data, the values of parameters in a PBK model will need to be derived from the results of *in silico* or *in vitro* experiments. Clearly, the accuracy of PBK models will be heavily reliant upon the quality of the model parameters, which often are not only tissue dependent but also chemical dependent.

To limit the extent of parameterization, it is useful to determine what a PBK model should contain at a minimum, and to build a decision tree (figure 3) for what a PBK model should minimally contain for defined situations (with respect to data-poor and data-rich chemicals). The most minimalistic model type, one-compartment models, parameterized with only protein binding and clearance data, have been developed and used to support chemical screening and prioritization (Rotroff et al., 2010; Wetmore et al., 2012, 2013, 2014; Tonnelier et al., 2012; Yoon et al., 2014; Wambaugh et al. 2015). Depending on the exposure route, a compartment representing the skin, intestine, or lung may need to be included in a model. If a compound is highly lipophilic, a fat compartment is required, and it may also be necessary for the model to describe uptake into the lymphatic system. Finally, depending on the hazard data available, additional compartments and biological processes may need to be added to the PBK model. Throughout development of the model, as more specific information is obtained on the chemical’s properties and mode of action (MoA), confidence is increased in the applicability of the models. A good strategy would be to begin with a generic model structure, then move to more specific models once knowledge is gained that indicates a unique biokinetic behaviour. In using a simple model, it is possible that a key kinetic pathway specific to a given target chemical will not be taken into consideration. To address this issue, a database of all known ADME/TK processes, such as cell uptake (capturing the role of transporters), metabolism, and efflux, could be developed to help new modellers consider which processes might need to be included for a specific purpose.

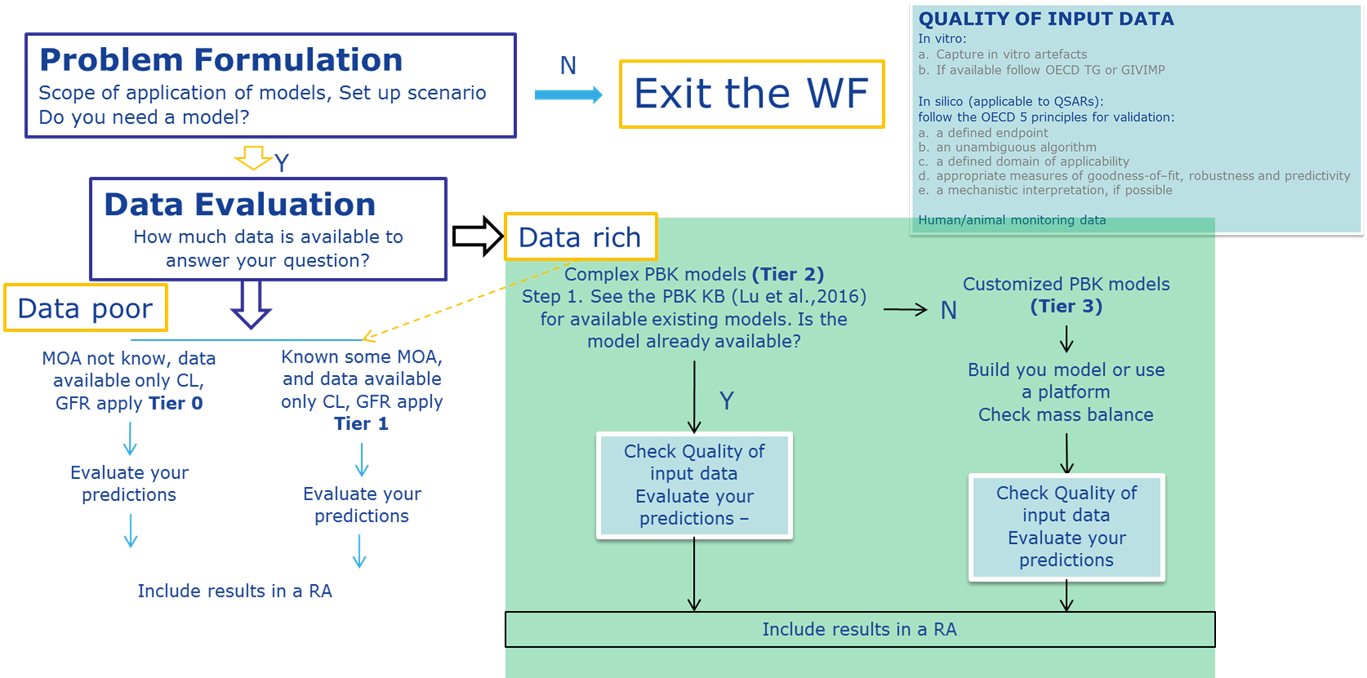


Figure 3. A schematic decision tree to decide what tier of PBK model to apply when encountering data-poor or data-rich chemicals during model parameterization and based on problem formulation.

Membrane transporters influence the ADME processes of various endogenous and exogenous compounds (Klaassen and Aleksunes, 2010; SOLVO, 2017). In recent decades, the pharmaceutical field has placed considerable effort into the study of transporters affecting drug disposition, therapeutic efficacy, and adverse outcomes, but little is known in regards to transporter effects on environmental chemicals (Clerbaux et al., 2018). Transporters can play a significant role in chemical distribution. As such, integration of membrane transporter experimental data during parameterization of several types of computational models (e.g., QSAR, pharmacophore, and PBK models), through use of platforms like SimCyp, PKSim, or GastroPlus, will enable better understanding of chemical/drug disposition (Clerbaux et al., 2018).

Protein binding in plasma functions to influence the partitioning of endogenous and exogenous compounds from the blood into the tissues. Plasma protein binding is related to lipophilicity, as binding becomes greater with more lipophilic chemicals, thus sequestering such chemicals and lowering their bioavailability. A common method for estimating plasma protein binding *in vitro* is the rapid equilibrium method, which involves measurement of chemical transport across a dialysis membrane with a high surface area-to-volume ratio within a Teflon-lined plate well (Waters et al., 2008).

Metabolism can be an important feature to include in a model, especially when a metabolite is assumed or known to be the toxic moiety. Both *in vitro* and *in silico* methods can be informative in providing predictions for metabolism and clearance. Kirchmair et al (2015) reviewed software for predicting a range of metabolism properties. The focus of these *in silico* tools is mainly the estimation of the qualitative nature of the metabolites (i.e., which metabolites are formed based on the parent compound’s molecular structure) and seldom allows for estimation of rate constants. A common criticism of software for predicting metabolites is the tendency for over-prediction: theoretically possible metabolites are not differentiated from those that occur experimentally. Some software platforms have attempted to address this issue through inclusion of filtering rules. For example, in order to reduce over-prediction within the Meteor Nexus software (Lhasa Ltd, Leeds), Marchant et al (2017) describe a process whereby k-nearest neighbor analysis is combined with expert knowledge of biotransformation to reduce the over-prediction of metabolites. Such *in silico* models do not predict efflux of metabolites.

*In vitro* data for metabolism may be generated using tissue slices, organ (e.g., liver) homogenates, cell lines, spheroids, or (sub)cellular fractions (such as microsomes, baculosomes, S9, and cytosol), where metabolism is measured as loss of the parent compound or production of metabolite(s). It should be noted that if metabolism occurs very slowly, it may not be detected in a short-term *in vitro* assay. If a chemical is known to be predominantly excreted unchanged in urine, then metabolism is less relevant to the model. However, if metabolism of a parent compound is thought to involve biliary excretion or excretion via the bile, then a model including such elimination pathways is necessary, by first determining which pathways of elimination are most relevant to the target chemical. *In silico* and *in vitro* models have also been developed to predict different processes involved in elimination. These include *in silico* models for total clearance (Lombardo et al., 2014) and metabolism (Pirovano et al., 2015) and *in vitro* models for biliary excretion (Ghibellini et al., 2006). However, more work is required to develop models for elimination, and the applicability domain for existing models should be carefully considered before application to a wider range of chemicals. A current limitation is that there are no (OECD) guideline(s) addressing *in vitro* methods to determine kinetic parameters, other than the guideline on Skin Absorption (OECD TG 428). In the absence of standardized methods for generating *in vitro* parameters to calibrate PBK models, it is important that *in vitro* metabolism data or data describing transporters are produced according to the new OECD good *in vitro* method practice (GIVIMP)[[3]](#footnote-3). The GIVIMP document is meant to serve as technical guidance on generating and applying quality data through good scientific and quality practices, to support the regulatory human safety assessment of chemicals using *in vitro* methods.

Model parameters can also be derived computationally. Within the literature, a vast number of *in silico* predictive models for ADME properties have been published, including models for skin and gastro-intestinal uptake, volume of distribution, tissue partitioning, and plasma protein binding. Bessems et al. (2014) provides a general overview of the currently available *in vitro* and *in silico* methods for human ADME and the gaps and challenges faced. Mostrag-Szlichtyng et al (2010) provide an extensive review specifically of *in silico* tools (i.e., QSAR models and software) for prediction of ADME properties that are relevant to PBK model building. More recently, Patel et al (2018) have collated and assessed the quality of over 80 models for 31 absorption-, distribution-, and excretion-related endpoints (Patel et al., 2018).

Finally, toxicodynamic data derived from *in vitro* toxicity tests are typically based on nominal concentrations of substances, which may contain significant errors due to the loss of biological, physical, and chemical processes in such tests. An *in vitro* biokinetic study plays a significant role in translating a nominal concentration used in *in vitro* systems to the actual level of cell exposure producing the effect. Several methodologies can be applied to address such a relationship, such as *in vitro* fate and transport mass balance models recently developed by several research teams (Kramer 2010a, 2010b; Armitage et al., 2014; Fischer et al., 2017; Zaldivar Comenges et al., 2017).

**2. Availability of modelling platforms**

Currently, several open source modelling platforms, such as IndusChemFAte (Cefic LRI, http://cefic-lri.org/toolbox/induschemfate/), High-Throughput Toxicokinetics (httk)-r package (Wambaugh et al., 2018, https://cran.r-project.org/web/packages/httk/index.html), MEGEN-RVis (Loizou et al, 2011; https://megen.useconnect.co.uk/), PLETHEM (http://www.scitovation.com/plethem.html), MERLIN-EXPO (Ciffroy et al., 2016b; Suciu et al., 2016; https://merlin-expo.eu/), and PK-Sim ([www.systems-biology.com](http://www.systems-biology.com)), and license-based platforms such as GastroPlus ([www.simulations-plus.com](http://www.simulations-plus.com)) and SimCyp (<https://www.certara.com>), are available to individuals possessing varying degrees of expertise in PBK modeling. These platforms provide different computational tools that allow non-programmers to develop and run model simulations with varying options to gain a better understanding of model behavior, which is essential for interpretation of model output. The PBK models run from these platforms can be parameterized using *in vitro* or *in silico* data. However, programmers or users with modeling skills can also use R, MATLAB, and Berkeley Madonna software to develop customized PBK models, and to support the generation of innovative modeling components, which might otherwise not be generated through use of the more-structured commercial platforms.

A concern for the use of open source modelling platforms, as compared to use of their proprietary counterparts, is the lack of sustainable resources and funding that are needed for further development and maintenance of those platforms. While most of these platforms are initiated by a research grant, upon completion of the project, the developers are often unable to find other funding sources to maintain it. In order for a modelling platform to remain sustainable, it is essential to maintain access to the model’s equations, so that these can be easily coded later. Sustainability also depends on the ability of model updates to be communicated to end-users. Establishment of an open source library as a repository for all available model information, including a peer review process, is strongly recommended.

**Salient Features: Integrating NG-PBK modelling with toxicodynamic endpoints**

There is high value in the use of PBK models to predict internal target tissue doses for risk assessment applications, based on the assumption that a similar tissue response arises from an equivalent target tissue dose, rather than the external dose, across different exposure conditions. In addition, toxicodynamic processes that that are interpreted in a high-throughput context from *in vitro* dose–response data can be integrated with PBK models, to link external exposure concentrations to target tissue doses to adverse endpoints. Such integration allows for support of several risk assessment extrapolations, such as QIVIVE and reverse dosimetry approaches. Examples of PBK/TD models are reported in table 3 of Punt et al., 2011. However, the application of PBK models in risk assessment requires proper evaluation of model purpose, model assumptions and structure, mathematical representation, parameter estimation, computer implementation, and predictive capacity. The topic of model evaluation will be captured in the next chapter.

**Salient Features: Model evaluation- assessing the validity and credibility of PBK models that rely only upon non-animal data**

A question that often arises is “*How can we trust a PBK model prediction if there are no in vivo data to evaluate the simulation, and how can the model gain credibility?”*

The following approaches could be applied and are described in further detail below: 1) read-across; 2) micro-scale systems; 3) pragmatic conservative scenario approach; 4) credibility matrix; 5) the reliability of dose metric predictions provided with uncertainty and sensitivity analyses (WHO 2010); and 6). population characteristics and virtual population libraries.

1. **Read-across**

For those cases in which *in vivo* data exist for one chemical, a read across approach[[4]](#footnote-4) may be applied to parameterize models for other chemicals (Schultz et al., 2015). For example, if a valid PBK model exists for chemical A (source chemical), and chemical B (target chemical) lacks any *in vivo* data and has been shown to be similar in structure to chemical A, then the same parameterized PBK model structure/code and *in vivo* data for chemical A can be used for chemical B. This read-across approach has been demonstrated by case studies applying the PBK Knowledgebase developed by Lu et al. (2016). Alternatively, if parameterization of the PBK model using available *in vitro* or *in silico* data for chemical B is possible, predictions can be compared to output from the model for chemical A based on *in vivo* data, in order to evaluate the PBK model for chemical B. When using such a model based on similarity between different chemicals, the influence of chemical-specific properties mediating ADME behaviour (e.g., log P, specific functional groups) should be carefully considered.

1. **Micro-scale systems**

Microscale systems, such as human-on-a-chip technology, could potentially be applied to measure and predict kinetics and whole body response to substances (Sung et al., 2014), thus aiding in evaluation and increased confidence in NG-PBK models. However, the limitations of these novel microscale systems should be carefully considered. For example, flow rates from model systems are often not scaled down in a similar manner as tissue volumes, thus rendering interpretation of the data difficult for PBK model applications.

1. **Pragmatic conservative scenario approach**

When *in vivo* data are lacking for model evaluation, a pragmatic conservative scenario could be followed in order to derive the most conservative estimate for risk assessment. For NG-PBK modeling, such an approach needs to be designed in such a way that the structure and input of the model is likely to lead to an overestimation of the internal concentration. This can be achieved by including uncertainty factors in the input parameters of the model. A worst-case estimate for absorption can for example be set to 100%. Other input parameters, such as metabolic clearance can be set to a value that is a certain extent lower than that measured for *in vitro* rates. To define the conservative boundaries around each input parameter, the uncertainties of each *in vitro* or *in silico* input method need to be globally identified.

1. **Credibility matrix**

There is a need to develop a framework for establishing the credibility of PBK models in support of risk assessment applications. As a first requirement for credibility, PBK models should be biologically plausible. Often, modellers or mathematicians exclude a number of biologically-relevant processes because these processes are considered to have no bearing on the model results and because models should be kept as simple as possible to allow for a desired outcome. However, such assumptions must always be discussed and agreed upon with biologists and toxicologists, to prevent the omission of critical biological and toxicological steps or key events. Good documentation of model assumptions is critical for modelers to demonstrate the credibility of their models to reviewers and users, and visualization is a key feature when dealing with communication of these models. The recent EFSA uncertainty guidance document provides a reporting table for listing and evaluating model uncertainties (EFSA 2018).

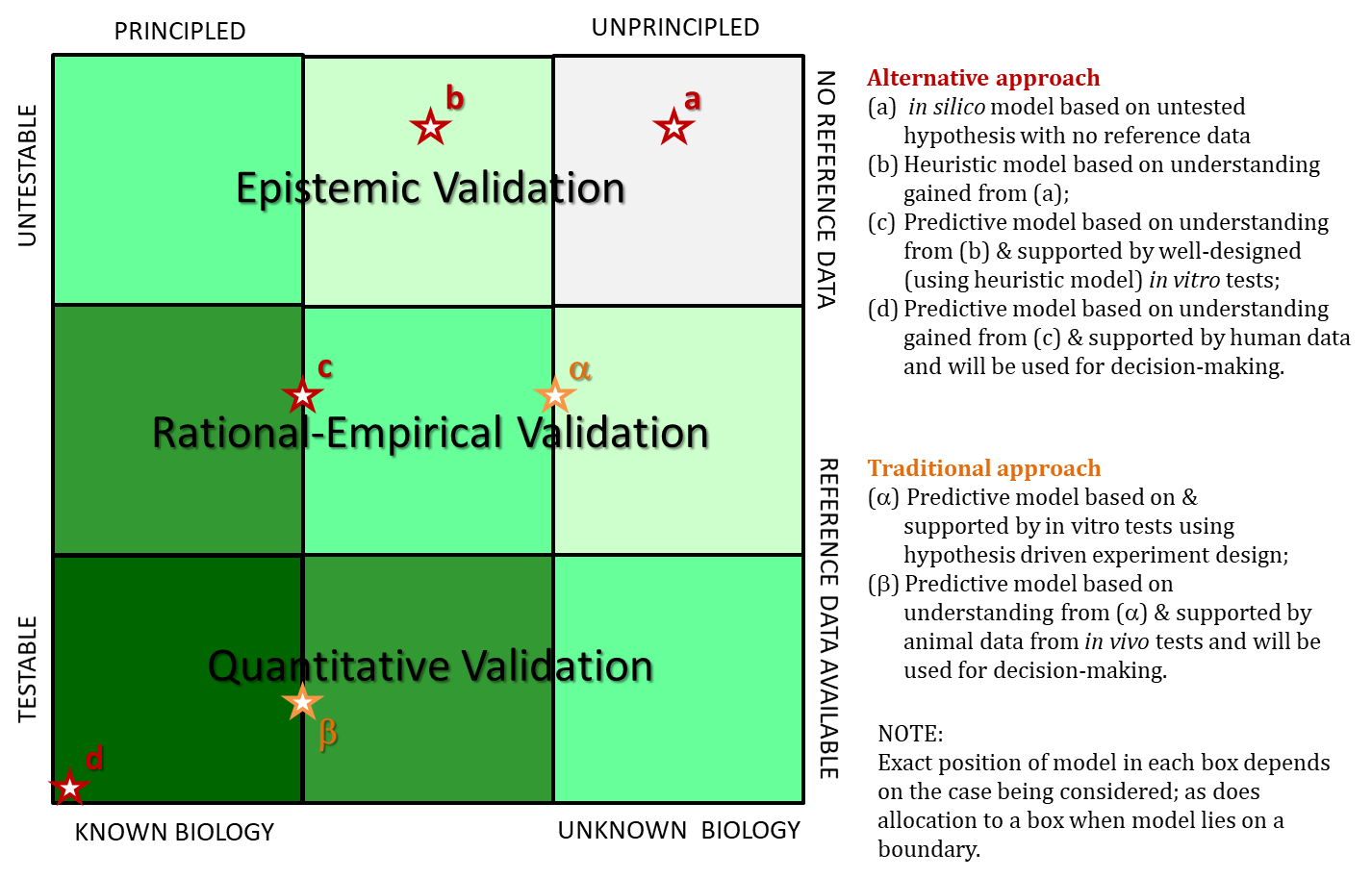
From the 2016 EURL ECVAM workshop, the following graphical representation and application of the “credibility matrix by Patterson & Whelan” has been proposed. The matrix, shown in figure 4, allows for locating a specific model type based on the information available, i.e. whether a model is principled and testable, as well as knowledge of the biology and the availability of data, which should aid in systematically evaluating model credibility (Patterson & Whelan, 2017). If a model falls in the bottom left region (testable and with full knowledge), confidence and credibility around the model is likely high. However, if a model falls in the top right region of the matrix (not testable and without any knowledge of the system biology), confidence in the model is likely low due to the uncertainties associated with it. In other words, regulators are unlikely to trust model types found in the top right region of the matrix when making decisions. The question is, to what degree a PBK model would need to be placed towards the bottom left corner to attain sufficient credibility for regulators. In some sense, testable models do not really predict, but provide an estimate to compare against available data in a retrospective fashion.

The proposed framework should lay out the requirements for validating models with different degrees of knowledge and testability (e.g., quantitative validation), which could aid in quantifying the uncertainty currently existing with animal models, and which can help regulators assess whether models developed through *in vitro* and *in silico* NAMs can be equally reliable, or even more so, compared to current risk assessment approaches. Biological systems, by nature, are complex networks operating under simple rules that can be described by non-linear dynamic processes, and which exhibit non-trivial emergent and self-organizing behavior. As a result, a measured value might represent a particular, and perhaps unknown, state of a system, which makes its use, as a comparator for a predicted value, challenging. To handle such issues, approaches that operate on experience-based validation are required. Ideally, these, approaches would capture the diversity of experiences to establish generic digital twins, which are couplings of validated models with their real-world datasets (see Patterson et al., 2016).

There is disagreement amongst modellers as to the meaning of the terms model evaluation, verification, and validation; for instance, EMA has shifted to use of the word “qualification”. Regardless of which term is more appropriate, the analytical purpose is to ensure that the model is appropriate for the task at hand, and that its predictions are a reasonable representation of reality. Once confirming that the model is a reasonable representation of reality for the intended purpose, several analyses may be used to “validate” a model, including sensitivity analysis, robustness analysis[[5]](#footnote-5), assumption justification, model argumentation, structured calibration, predictive performance, proper scoring rules, and relation to reality. To “verify” a model, the model scope should be revisited and the model equations and code reviewed. The following key elements were suggested by the 2016 EURL ECVAM workshop participants to achieve model credibility by regulators (Paini et al., 2017a):

* Understand the model;
* Understand the data underpinning the model;
* State clearly the assumptions and hypothesis encoded;
* Consider the gap between the model and reality, based on available observations.

This last item can be a description of what is lacking in the model. The outcomes of sensitivity analyses can be used to explain some model deficits. One possible approach, as opposed to the statement in the introduction regarding developing the simplest model, would be to start with a more complex model and then remove parameters to which the predictions are not sensitive. The potential problem with this approach is that when there are many parameters with large uncertainties, they may introduce a great deal of variation into the uncertainty analysis.

****

**Figure 4.** Credibility matrix showing comparative loci for a model based on traditional *in vivo* data-based approaches and for a model based on an alternative approach (i.e., NAM). The rationale for the locations of the model types, indicated by stars and letters, are given in the side-bar legend. For example, *in silico* models placed at the top right, might consist of a simple model ‘a’ based on a limited set of data, for instance in a QSAR. This leads to a more sophisticated, but still heuristic, model ‘b’ based on the understanding gained from model ‘a’. The predictions from models ‘a’ and ‘b’ are used to design *in vitro* tests that enable the development of model ‘c’, which can be validated using the rational-empirical approach, thus enhancing its credibility. Finally, this leads to the development of clinical studies and model ‘d’, supported by its predecessors and quantitatively validated or confirmed using clinical data. This places model ‘d’ in the bottom left corner, as a model whose predictions stakeholders, including regulators, practitioners, and patients, will likely use to make decisions (adapted from Paini et al., 2017a, proposed by Patterson and Whelan 2017).

1. **Reliability of dose metric predictions (model testing, uncertainty, and sensitivity)**

In 2010, the World Health Organization (WHO) reported the level of confidence needed to gain credibility in a PBK model intended for risk assessment (WHO, 2010). The degree of confidence in a PBK model’s predictions depends upon how well the model has been tested against real data and whether adequate sensitivity and uncertainty analyses have been conducted, in order to support the reliability of predictions (WHO, 2010). In our case, we could lack the “real data” required to evaluate model predictions for the purpose of validation. However, reporting of adequate sensitivity and uncertainty is certainly relevant and encouraged. Tables providing guidance in reporting results of uncertainty and sensitivity analyses have been provided in the WHO 2010 article, as a tool to better document the evaluation of model predictions (from WHO 2010; Meek et al. 2013).

1. **Population characteristics and virtual population libraries**

PBK models act to support several purposes for both developers and regulators. One of the most popular, among recent applications, has been the prediction of drug-drug interactions and investigation of drug dosing for therapeutic purposes in both pediatric and adult patients with rare diseases, in an effort to develop libraries of "virtual patients" or “virtual populations” for clinical trials. Several ongoing European Horizon 2020 projects are developing such "virtual population libraries"[[6]](#footnote-6), in addition to "data integration and data-driven in-silico models for transitioning personalized medicine towards a European standardization framework", with focus of their impact on harmonization of health disease data integration and data-driven in-silico models in Europe. This effort was undertaken to better capture heterogeneity in human. Important aspects of human heterogeneity include interindividual variations in lifestyle, health status (immunosuppressed, disease patient) genetic polymorphism (gene expression), physiology (uptake rate), biochemistry and molecular biology (Mclanahan et al., 2012) with respect to age as well. These factors will interact and influence the chemical ADME and biokinetic and toxicodynamics within the body. Parameters in a PBK model have a direct biological correspondence, providing a useful framework for determining the impact of observed variations in physiological and biochemical factors on the population variability in dosimetry within the context of a risk assessment for a particular chemical (Clewell and Andersen, 1996; Price et al., 2003; Mclanahan et al., 2012). In addition, integration of genetic information from –omics studies will enhance predictions for precise and personalized medicine. Applications for predicting specific populations, such as pediatrics, have been increasing in their development and use (Leong et al., 2012). Advances in in vitro and in silico tools and technologies are behind the success of generic PBK model tools in the pharmaceutical field. These models can reproduce clinical observations and, more importantly, simulate (i.e., predict) untested clinical outcomes, allowing for evaluating the effects of intrinsic (e.g., organ dysfunction, age, genetics, etc.) and extrinsic (e.g., drug-drug interactions) factors, alone or in combination, on drug exposure. There are several areas that are considered to present current challenges in accepting model-informed drug development, which can also provide insight into the necessary acceptance criteria for PBK model-based drug development. Among those criteria, most noteworthy is that the adequacy of submitted PBK models is to be based on their intended purposes at different stages of drug development (Paini et al., 2017a). That is, determination of whether a model is fit-for-purpose and the need to identify and transparently communicate the knowledge gaps. EMA and US FDA published a draft document in 2016 as guidance on the qualification and reporting of physiologically based pharmacokinetic (PBPK) modelling and simulations (EMA, 2016; US FDA 2016). The aim of this guideline is to describe the expected content that should be included in PBK modelling and simulation reports during regulatory submission, including applications for authorization of medicinal products, pediatric investigation plans, and clinical trial applications. This also includes the documentation needed to support the qualification of a PBK platform for an intended use, such as results of sensitivity and uncertainty analyses.

**Next steps and perspectives**

With an increasing demand for application of alternative methods within the risk assessment framework, the need for development of higher throughput NG-PBK models has also increased. A guidance document for GMP for PBK modelling could also be extended to other types of *in silico* biokinetic models, such as *in vitro* mass balance models (Armitage et al., 2014; Zaldivar Comenges et al., 2017). Existing guidance documents (WHO, 2010 and EPA, 2006), and those documents of EFSA (2014), and European Committee for Standardization (CEN, 2015), that are less PBK-specific, require updating with respect to the current trends, due to the continuous evolution in science and risk assessment. The recent United States Food and Drug Administration (US FDA, 2016) and European Medicine Agency (EMA, 2016) guidelines are the first that open up the possibility to submit non-animal PBK model results for drug dossier submission and provide excellent examples that other agencies could follow. At the same time, the OECD is working on a guidance document for the characterization, validation, and reporting of physiologically based models for regulatory applications that should be ready in 2019, and which attempts to set principles for NG-PBK model validation.

However, the challenge remains of making appropriate use of *in vitro* data or *in silico* predictions when 1) building these models; 2) interpreting model outputs and integrating the outputs with other sources of information for risk assessment purposes; and 3) attempting to gain model credibility by underlining all uncertainties and assumptions when *in vivo* human data is unavailable for proper model evaluation. The uncertainty and variability associated with PBK models, and the proposed GMP (Loizou et al., 2008), should be further developed and should include guidance for PBK models built using NGMs to measure ADME properties. The use of a matrix in the new risk assessment paradigm, to underline and quantify the uncertainty associated with NG-PBK models, compared to models based on *in vivo* animal data, would be desirable.

Several standardized decision trees could be developed to guide modellers in their construction of a PBK model in the absence of *in vivo* data for calibration, and to guide risk assessors in the application and interpretation of PBK models. For instance, PBK-predicted internal dose metrics vs. *in vitro* PoD from toxicity testing could be taken into account, along with *in vitro* results linking to *in vivo* adverse outcomes for a tiered assessment, perhaps through application of the traditional and internal TTC approach (Kroes et al., 2007; Worth et al., 2012). With the need for several international working groups to further develop such documentation, communication is required among these groups to ensure compatibility of *in vitro* kinetic and dynamic methods with PBK models, in addition to communication with regulators to fit the total risk-assessment framework. It should be noted that for such communication to be achieved, funding would be necessary.

There remains a need to create a community to address issues with human ADME/TK and NG-PBK models, such as the development of criteria for model construction and model evaluation. A group of scientists across the academic, industrial, and governmental landscapes should be available and willing to establish a peer review system for PBK models. Criteria should exist to select those individuals that will review the models, and templates and check lists should be provided to assist in the review process. A public repository is needed for PBK models that have been built and/or peer reviewed, and once this repository is developed, relevant documentation can be introduced from an independent peer review to support model credibility. Such a repository is in line with the work reported in Lu et al., (2016) and will allow for the curation of more case studies and the creation of libraries of *ad hoc* PBK models that could be used for training purposes. Additionally, this repository will facilitate risk assessment approaches applying PBK models and IVIVE, and communicate to decision makers more efficiently the current state of science regarding the use of animal-free models in regulatory applications. Perspectives from the various industrial stakeholders (e.g. pharmaceutical, food safety, agricultural, and personal care product industries) also need to be communicated, to provide greater insight of current practice and understanding of future needs of these sectors, to enable promotion of best practices.

Application of NG-PBK models, in the context of exposure in specific population of patients, would be extremely valuable in the generation of virtual population/patient libraries. These libraries would enable clinical trials to entail populations with a greater number of “virtual” individuals, which might not otherwise be possible to conduct with a limited number of real persons. Additionally, these libraries would introduce populations more rarely encountered, such as those possessing enzyme polymorphisms that exert a greater influence on drug-drug interactions or those with rare genetic diseases or health abnormalities.

Finally, it is recommended to establish a means for training new modellers and risk assessors. Such training, which can be provided with specific courses or as a continuous education course within scientific conferences, will focus on PBK model development, evaluation, and application. Though several challenges still remain, the suggestions and steps presented in this work provide a path towards gaining acceptance of NG-PBK models in regulatory practices.

In conclusion, to facilitate the development and use of NG-PBK models, which do not rely on animal *in vivo* data, and their acceptance in the regulatory domain, the following are recommended:

i) development of more transparent, accessible, and user-friendly software platforms that facilitate development and application of PBK models by a community of users;

ii) development of a single resource to inform new developments in *in silico* and *in vitro* approaches that may be used to provide data for model development;

iii) development and refinement of existing web applications and PBK model platforms that have the ability to conduct IVIVE and reverse dosimetry in an automated manner;

iv) knowledge sharing initiatives that allow members of the regulatory community, such as risk assessors and risk managers, to become familiar with relevant PBK model information, while model developers gain a better understanding of regulatory needs;

v) good modelling practices and harmonized guidelines for reporting the steps taken during model development, evaluation, and application, with respect to NG- PBK models. This would include the use of a clear and common terminology.

**Acknowledgements**

The authors would like to provide a special thanks to E. Ahs and G. Tosiou for logistical and practical support during the workshop. The authors thank S. Belz, R. Corvi, P. Prieto-Peraita, A. Richarz, and M. Whelan of the JRC for contributing to discussions during the workshop.

**Funding information**

This work was supported by the European Commission Joint Research Centre (JRC), Directorate F –Health, Consumers and Reference Materials and European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM), Ispra, Italy. Funding for Dr. Leonard was provided by the Oak Ridge Institute for Science and Education Research Participation Program at the US EPA.

**Disclaimer**

The views expressed in this paper are those of the authors and do not necessarily reflect the views of the author/s institutions. Authors declare no conflicts of interest.

**References**

1. Altenpohl A, Ciffroy P, Paini A, Radovnkovic A, Suciu NA, Tanaka T, Tediosi A,,Verdonck F (2018). Standard documentation of exposure models: Merlin-Expo case study, Handbook of Environmental Chemistry, Volume 57, Pages 59-76.
2. Andersen & Krishnan (2010). Quantitative Modeling in Toxicology: An Introduction Book Editor(s): Dr. Kannan Krishnan Dr Melvin E. Andersen. Wiley Online Library. First published: 30 March 2010 <https://doi.org/10.1002/9780470686263.ch1>
3. Armitage JM, Wania F, Arnot JA (2014). Application of mass balance models and the chemical activity concept to facilitate the use of in vitro toxicity data for risk assessment. Environ. Sci. Technol., 48 (16), pp. 9770-9779.
4. Barton HA, Bessems J, Bouvier d'Yvoire M, Buist H, Clewell III H, Gundert-Remy U, et al. (2009). Principles of Characterizing and Applying Physiologically-Based Pharmacokinetic and Toxicokinetic Models in Risk Assessment. IPCS project on the Harmonization of Approaches to the Assessment of Risk from Exposure to Chemicals.
5. Barton HA, Chiu WA, Setzer RW, Andersen ME, Bailer AJ, Bois FY, et al. (2007). Characterizing uncertainty and variability in physiologically-based pharmacokinetic (PBPK) models: state of the science and needs for research and implementation. Toxicol Sci, 99(2), 395-402.
6. [Bell SM](https://www.ncbi.nlm.nih.gov/pubmed/?term=Bell%20SM%5BAuthor%5D&cauthor=true&cauthor_uid=29203341), [Chang X](https://www.ncbi.nlm.nih.gov/pubmed/?term=Chang%20X%5BAuthor%5D&cauthor=true&cauthor_uid=29203341), [Wambaugh JF](https://www.ncbi.nlm.nih.gov/pubmed/?term=Wambaugh%20JF%5BAuthor%5D&cauthor=true&cauthor_uid=29203341), [Allen DG](https://www.ncbi.nlm.nih.gov/pubmed/?term=Allen%20DG%5BAuthor%5D&cauthor=true&cauthor_uid=29203341), [Bartels M](https://www.ncbi.nlm.nih.gov/pubmed/?term=Bartels%20M%5BAuthor%5D&cauthor=true&cauthor_uid=29203341), [Brouwer KLR](https://www.ncbi.nlm.nih.gov/pubmed/?term=Brouwer%20KLR%5BAuthor%5D&cauthor=true&cauthor_uid=29203341), [Casey WM](https://www.ncbi.nlm.nih.gov/pubmed/?term=Casey%20WM%5BAuthor%5D&cauthor=true&cauthor_uid=29203341), [Choksi N](https://www.ncbi.nlm.nih.gov/pubmed/?term=Choksi%20N%5BAuthor%5D&cauthor=true&cauthor_uid=29203341), Ferguson SS, [Fraczkiewicz G](https://www.ncbi.nlm.nih.gov/pubmed/?term=Fraczkiewicz%20G%5BAuthor%5D&cauthor=true&cauthor_uid=29203341), [Jarabek AM](https://www.ncbi.nlm.nih.gov/pubmed/?term=Jarabek%20AM%5BAuthor%5D&cauthor=true&cauthor_uid=29203341), [Ke A](https://www.ncbi.nlm.nih.gov/pubmed/?term=Ke%20A%5BAuthor%5D&cauthor=true&cauthor_uid=29203341), [Lumen A](https://www.ncbi.nlm.nih.gov/pubmed/?term=Lumen%20A%5BAuthor%5D&cauthor=true&cauthor_uid=29203341), [Lynn SG](https://www.ncbi.nlm.nih.gov/pubmed/?term=Lynn%20SG%5BAuthor%5D&cauthor=true&cauthor_uid=29203341), [Paini A](https://www.ncbi.nlm.nih.gov/pubmed/?term=Paini%20A%5BAuthor%5D&cauthor=true&cauthor_uid=29203341), [Price PS](https://www.ncbi.nlm.nih.gov/pubmed/?term=Price%20PS%5BAuthor%5D&cauthor=true&cauthor_uid=29203341), [Ring C](https://www.ncbi.nlm.nih.gov/pubmed/?term=Ring%20C%5BAuthor%5D&cauthor=true&cauthor_uid=29203341), [Simon TW](https://www.ncbi.nlm.nih.gov/pubmed/?term=Simon%20TW%5BAuthor%5D&cauthor=true&cauthor_uid=29203341), [Sipes NS](https://www.ncbi.nlm.nih.gov/pubmed/?term=Sipes%20NS%5BAuthor%5D&cauthor=true&cauthor_uid=29203341), [Sprankle CS](https://www.ncbi.nlm.nih.gov/pubmed/?term=Sprankle%20CS%5BAuthor%5D&cauthor=true&cauthor_uid=29203341), [Strickland J](https://www.ncbi.nlm.nih.gov/pubmed/?term=Strickland%20J%5BAuthor%5D&cauthor=true&cauthor_uid=29203341), [Troutman J](https://www.ncbi.nlm.nih.gov/pubmed/?term=Troutman%20J%5BAuthor%5D&cauthor=true&cauthor_uid=29203341), [Wetmore BA](https://www.ncbi.nlm.nih.gov/pubmed/?term=Wetmore%20BA%5BAuthor%5D&cauthor=true&cauthor_uid=29203341), [Kleinstreuer NC](https://www.ncbi.nlm.nih.gov/pubmed/?term=Kleinstreuer%20NC%5BAuthor%5D&cauthor=true&cauthor_uid=29203341). (2018) In vitro to in vivo extrapolation for high throughput prioritization and decision making. [Toxicol In Vitro.](https://www.ncbi.nlm.nih.gov/pubmed/29203341) 2018 Mar;47:213-227.
7. Bessems JG, Loizou G, Krishnan K, Clewell HJ, Bernasconi, C, Bois FY, Coecke S, Collnot EM, Diembeck W, Farcal et al. (2014). PBTK modelling platforms and parameter estimation tools to enable animal-free risk assessment: recommendations from a joint EPAA--EURL ECVAM ADME workshop. Regul Toxicol Pharmacol. 68(1):119-139.
8. Bischoff KB, Dedrick RL (1968). Thiopental pharmacokinetics. J Pharm Sci. 57(8):1346-1351.
9. Bischoff KB, Dedrick RL, Zaharko DS, Longstreth JA (1971). Methotrexate pharmacokinetics. J Pharm Sci. 60(8):1128-1133.
10. Blaauboer B, Bayliss MK, Castell J, Evelo CTA, Frazier JM, Groen K, Gulden M, Guillouzo A, Hissink, AM, Houston B, Johanson G, de Jongh J, Kedderis GL, Reinhardt CA, van de Sandt JJM, Semino G (1996). The use of biokinetics and in vitro methods in toxicological risk evaluation. The report and recommendations of ECVAM Workshop 15. ATLA 24:473-497.
11. Bouvier d'Yvoire M, Prieto P, Blaauboer BJ, Bois FY, Boobis A, Brochot C, Coecke S, Freidig A, Gundert-Remy U, Hartung T, et al. (2007). Physiologically-based Kinetic Modelling (PBK Modelling): meeting the 3Rs agenda. The report and recommendations of ECVAM Workshop 63. ATLA 35(6):661-671.
12. [Bois FY](https://www.ncbi.nlm.nih.gov/pubmed/?term=Bois%20FY%5BAuthor%5D&cauthor=true&cauthor_uid=27267299), [Ochoa JGD](https://www.ncbi.nlm.nih.gov/pubmed/?term=Ochoa%20JGD%5BAuthor%5D&cauthor=true&cauthor_uid=27267299), [Gajewska M](https://www.ncbi.nlm.nih.gov/pubmed/?term=Gajewska%20M%5BAuthor%5D&cauthor=true&cauthor_uid=27267299), [Kovarich S](https://www.ncbi.nlm.nih.gov/pubmed/?term=Kovarich%20S%5BAuthor%5D&cauthor=true&cauthor_uid=27267299), [Mauch K](https://www.ncbi.nlm.nih.gov/pubmed/?term=Mauch%20K%5BAuthor%5D&cauthor=true&cauthor_uid=27267299), [Paini A](https://www.ncbi.nlm.nih.gov/pubmed/?term=Paini%20A%5BAuthor%5D&cauthor=true&cauthor_uid=27267299), [Péry A](https://www.ncbi.nlm.nih.gov/pubmed/?term=P%C3%A9ry%20A%5BAuthor%5D&cauthor=true&cauthor_uid=27267299), [Benito JVS](https://www.ncbi.nlm.nih.gov/pubmed/?term=Benito%20JVS%5BAuthor%5D&cauthor=true&cauthor_uid=27267299), [Teng S](https://www.ncbi.nlm.nih.gov/pubmed/?term=Teng%20S%5BAuthor%5D&cauthor=true&cauthor_uid=27267299), [Worth A](https://www.ncbi.nlm.nih.gov/pubmed/?term=Worth%20A%5BAuthor%5D&cauthor=true&cauthor_uid=27267299) (2017). Multiscale modelling approaches for assessing cosmetic ingredients safety. [Toxicology.](https://www.ncbi.nlm.nih.gov/pubmed/?term=bois+paini+2016) 392:130-139.
13. CEN, European committee for standardization (2015). CEN Workshop on Standard documentation of large chemical exposure models (WS MERLIN-EXPO); CWA 16938 Brussels https://www.cen.eu/work/areas/chemical/Pages/WS-MerlinExpo.aspx
14. Ciffroy P, Altenpohl A, Fait G, Fransman W, Paini A, Radovnikovic A, Simon-Cornu M, Suciu N, Verdonck F (2016a). Development of a standard documentation protocol for communicating exposure models. Sci Total Environ 568, 557-565
15. Ciffroy P, Alfonso B, A. Altenpohl, Z. Banjac, J. Bierkens, C. Brochot, A. Critto, T. De Wilde, G. Fait, A. Tediosi, T. Fierens, J. Garratt, E. Giubilato, E. Grange, E. Johansson, A. Radomyski, K. Reschwann, N. Suciu, M. Van Holderbeke, F. Verdonck, A. Vlajic (2016b). Modelling the exposure to chemicals for risk assessment: a comprehensive library of multimedia and PBPK models for integration, prediction, uncertainty and sensitivity analysis — the MERLIN-expo tool. Sci. Total Environ. 568:770-784.
16. [Clerbaux LA](https://www.ncbi.nlm.nih.gov/pubmed/?term=Clerbaux%20LA%5BAuthor%5D&cauthor=true&cauthor_uid=30015123), [Coecke S](https://www.ncbi.nlm.nih.gov/pubmed/?term=Coecke%20S%5BAuthor%5D&cauthor=true&cauthor_uid=30015123), [Lumen A](https://www.ncbi.nlm.nih.gov/pubmed/?term=Lumen%20A%5BAuthor%5D&cauthor=true&cauthor_uid=30015123), [Kliment T](https://www.ncbi.nlm.nih.gov/pubmed/?term=Kliment%20T%5BAuthor%5D&cauthor=true&cauthor_uid=30015123), [Worth AP](https://www.ncbi.nlm.nih.gov/pubmed/?term=Worth%20AP%5BAuthor%5D&cauthor=true&cauthor_uid=30015123), [Paini A](https://www.ncbi.nlm.nih.gov/pubmed/?term=Paini%20A%5BAuthor%5D&cauthor=true&cauthor_uid=30015123). (2018) Capturing the applicability of in vitro-in silico membrane transporter data in chemical risk assessment and biomedical research. [Sci Total Environ.](https://www.ncbi.nlm.nih.gov/pubmed/30015123) 2018 Dec 15;645:97-108. doi: 10.1016/j.scitotenv.2018.07.122. Epub 2018 Jul 14.
17. Clewell, H. J., III, and Andersen, M. E. (1996). Use of physiologically based pharmacokinetic modeling to investigate individual versus population risk. Toxicology 111, 315–329.
18. Clewell III HJ, Andersen ME, Blaauboer BJ. (2008) On the incorporation of chemical-specific information in risk assessment. Toxicology Letters 180 (2008) 100–109
19. EFSA. (2014). Scientific opinion on good modelling practice in the context of mechanistic effect models for risk assessment of plant protection products. EFSA journal, 12(3): 3589
20. EFSA (2018). Guidance on Uncertainty in EFSA Scientific Assessment, http://www.efsa.europa.eu/en/efsajournal/pub/5123
21. EMA European Medicine Agency (2016). Draft “Guideline on the qualification and reporting of physiologically based pharmacokinetic (PBPK) modelling and simulation.” http://www.ema.europa.eu/docs/en\_GB/document\_library/Scientific\_guideline/2016/07/WC500211315.pdf.
22. Fischer FC, Henneberger L., König M., Bittermann K., Linden L., Goss K.U., Escher B.I. (2017) Modeling Exposure in the Tox21 in vitro bioassays Chem. Res. Toxicol., 5, pp. 1197-1208.
23. Ghibellini G, Leslie EM, & Brouwer KLR (2006). Methods to Evaluate Biliary Excretion of Drugs in Humans: an Updated Review. Mol Pharm. 3(3): 198–211.
24. Groothuis FA, Heringa MB, Nicol B, Hermens JL, Blaauboer BJ, and Kramer NI (2015). Dose metric considerations in in vitro assays to improve quantitative in vitro-in vivo dose extrapolations. Toxicology, 332, 30-40.
25. Kirchmair J, Göller AH, Lang D, Kunze J, Testa B, Wilson ID et al. (2015). Predicting drug metabolism: experiment and/or computation? (2015). Nature Reviews Drug Discovery, 14(6), 387-404.
26. Klaassen, CD., Aleksunes LM., (2010) Xenobiotic, bile acid, and cholesterol transporters. Pharmacol. Rev., 62 (1) , pp. 1-96.
27. Kramer NI, Busser FJ, Oosterwijk MT, Schirmer K, Escher BI, Hermens, JL (2010a). Development of a partition-controlled dosing system for cell assays. Chemical research in toxicology, 23(11), 1806-1814.
28. Kramer NI (2010b). Measuring, modeling, and increasing the free concentration of test chemicals in cell assays. Utrecht University.
29. Krishnan & Peyret (2009). Physiologically Based Toxicokinetic (PBTK) Modeling in Ecotoxicology J. Devillers (ed.), Ecotoxicology Modeling, Emerging Topics in Ecotoxicology: Principles, Approaches and Perspectives 2, DOI 10.1007/978-1-4419-0197-2 6, Springer Science+Business Media, LLC 2009
30. Kroes R, Renwick AG, Feron V, Galli CL, Gibney M, Greim H, Guy RH, Lhuguenot JC, van de Sandt JJM (2007). Application of the threshold of toxicological concern (TTC) to the safety evaluation of cosmetic ingredients. Food Chem. Toxicol. 45: 2533-2562
31. [Laroche C](https://www.ncbi.nlm.nih.gov/pubmed/?term=Laroche%20C%5BAuthor%5D&cauthor=true&cauthor_uid=30144470), [Aggarwal M](https://www.ncbi.nlm.nih.gov/pubmed/?term=Aggarwal%20M%5BAuthor%5D&cauthor=true&cauthor_uid=30144470), [Bender H](https://www.ncbi.nlm.nih.gov/pubmed/?term=Bender%20H%5BAuthor%5D&cauthor=true&cauthor_uid=30144470), [Benndorf P](https://www.ncbi.nlm.nih.gov/pubmed/?term=Benndorf%20P%5BAuthor%5D&cauthor=true&cauthor_uid=30144470), [Birk B](https://www.ncbi.nlm.nih.gov/pubmed/?term=Birk%20B%5BAuthor%5D&cauthor=true&cauthor_uid=30144470), [Crozier J](https://www.ncbi.nlm.nih.gov/pubmed/?term=Crozier%20J%5BAuthor%5D&cauthor=true&cauthor_uid=30144470), [Dal Negro G](https://www.ncbi.nlm.nih.gov/pubmed/?term=Dal%20Negro%20G%5BAuthor%5D&cauthor=true&cauthor_uid=30144470), [De Gaetano F](https://www.ncbi.nlm.nih.gov/pubmed/?term=De%20Gaetano%20F%5BAuthor%5D&cauthor=true&cauthor_uid=30144470), [Desaintes C](https://www.ncbi.nlm.nih.gov/pubmed/?term=Desaintes%20C%5BAuthor%5D&cauthor=true&cauthor_uid=30144470), [Gardner I](https://www.ncbi.nlm.nih.gov/pubmed/?term=Gardner%20I%5BAuthor%5D&cauthor=true&cauthor_uid=30144470), [Hubesch B](https://www.ncbi.nlm.nih.gov/pubmed/?term=Hubesch%20B%5BAuthor%5D&cauthor=true&cauthor_uid=30144470), [Irizar A](https://www.ncbi.nlm.nih.gov/pubmed/?term=Irizar%20A%5BAuthor%5D&cauthor=true&cauthor_uid=30144470), [John D](https://www.ncbi.nlm.nih.gov/pubmed/?term=John%20D%5BAuthor%5D&cauthor=true&cauthor_uid=30144470), [Kumar V](https://www.ncbi.nlm.nih.gov/pubmed/?term=Kumar%20V%5BAuthor%5D&cauthor=true&cauthor_uid=30144470), [Lostia A](https://www.ncbi.nlm.nih.gov/pubmed/?term=Lostia%20A%5BAuthor%5D&cauthor=true&cauthor_uid=30144470), [Manou I](https://www.ncbi.nlm.nih.gov/pubmed/?term=Manou%20I%5BAuthor%5D&cauthor=true&cauthor_uid=30144470), [Monshouwer M](https://www.ncbi.nlm.nih.gov/pubmed/?term=Monshouwer%20M%5BAuthor%5D&cauthor=true&cauthor_uid=30144470), [Müller BP](https://www.ncbi.nlm.nih.gov/pubmed/?term=M%C3%BCller%20BP%5BAuthor%5D&cauthor=true&cauthor_uid=30144470), [Paini A](https://www.ncbi.nlm.nih.gov/pubmed/?term=Paini%20A%5BAuthor%5D&cauthor=true&cauthor_uid=30144470), [Reid K](https://www.ncbi.nlm.nih.gov/pubmed/?term=Reid%20K%5BAuthor%5D&cauthor=true&cauthor_uid=30144470), [Rowan T](https://www.ncbi.nlm.nih.gov/pubmed/?term=Rowan%20T%5BAuthor%5D&cauthor=true&cauthor_uid=30144470), [Sachana M](https://www.ncbi.nlm.nih.gov/pubmed/?term=Sachana%20M%5BAuthor%5D&cauthor=true&cauthor_uid=30144470), [Schutte K](https://www.ncbi.nlm.nih.gov/pubmed/?term=Schutte%20K%5BAuthor%5D&cauthor=true&cauthor_uid=30144470), [Stirling C](https://www.ncbi.nlm.nih.gov/pubmed/?term=Stirling%20C%5BAuthor%5D&cauthor=true&cauthor_uid=30144470), [Taalman R](https://www.ncbi.nlm.nih.gov/pubmed/?term=Taalman%20R%5BAuthor%5D&cauthor=true&cauthor_uid=30144470), [van Aerts L](https://www.ncbi.nlm.nih.gov/pubmed/?term=van%20Aerts%20L%5BAuthor%5D&cauthor=true&cauthor_uid=30144470), [Weissenhorn R](https://www.ncbi.nlm.nih.gov/pubmed/?term=Weissenhorn%20R%5BAuthor%5D&cauthor=true&cauthor_uid=30144470)8, [Sauer UG](https://www.ncbi.nlm.nih.gov/pubmed/?term=Sauer%20UG%5BAuthor%5D&cauthor=true&cauthor_uid=30144470)25. (2018) Finding Synergies for 3Rs – Toxicokinetics and Read-Across: Report from an EPAA Partners’ Forum [Regul Toxicol Pharmacol.](https://www.ncbi.nlm.nih.gov/pubmed/30144470) 2018 Aug 23;99:5-21
32. Leong, R., Vieira, M. L. T., Zhao, P., Mulugeta, Y., Lee, C. S., Huang, S.-M. and Burckart, G. J. (2012). Regulatory Experience With Physiologically Based Pharmacokinetic Modeling for Pediatric Drug Trials. Clinical Pharmacology & Therapeutics, 91(5): 926–931. doi:10.1038/clpt.2012.19
33. Loizou GD, Spendiff M, Barton HA, Bessems J, Bois FY, Bouvier M et al. (2008). Development of Good Modelling Practice for Physiologically Based Pharmacokinetic Models for Use in Risk Assessment: The First Steps. Reg. Toxicol. Pharmacol., 50(3), 400-411.
34. Loizou GD & Hogg A (2011). MEGen: A Physiologically Based Pharmacokinetic Model Generator. Frontiers in Pharmacology, 2 (56), 1-14, 10.3389/fphar.2011.00056.
35. Louisse J, Bosgra S, Blaauboer BJ, Rietjens IM, Verwei M (2015). Prediction of in vivo developmental toxicity of all-trans-retinoic acid based on in vitro toxicity data and in silico physiologically based kinetic modeling. Arch Toxicol. 89(7):1135-1148.
36. [Louisse J](https://www.ncbi.nlm.nih.gov/pubmed/?term=Louisse%20J%5BAuthor%5D&cauthor=true&cauthor_uid=27768849), [Beekmann K](https://www.ncbi.nlm.nih.gov/pubmed/?term=Beekmann%20K%5BAuthor%5D&cauthor=true&cauthor_uid=27768849), [Rietjens IM](https://www.ncbi.nlm.nih.gov/pubmed/?term=Rietjens%20IM%5BAuthor%5D&cauthor=true&cauthor_uid=27768849) (2017) Use of Physiologically Based Kinetic Modeling-Based Reverse Dosimetry to Predict in Vivo Toxicity from in Vitro Data. C[hem Res Toxicol.](https://www.ncbi.nlm.nih.gov/pubmed/27768849) 17; 30(1):114-125.
37. Lowenthal DT, Briggs WA, Levy G (1974). Kinetics of salicylate elimination by anephric patients. J Clin Invest. 54(5):1221-1226.
38. Lu J, Goldsmith MR, Grulke CM, Chang DT, Brooks RD, Leonard JA, & Johnson J.(2016). Developing a physiologically-based pharmacokinetic model knowledgebase in support of provisional model construction. PLoS Comput. Biol., 12 (2).
39. Marchant CA, Rosser, EM, Vessey JD (2017). A k‐Nearest Neighbours Approach Using Metabolism‐related Fingerprints to Improve In Silico Metabolite Ranking. Molecular informatics, 36(3).McLanahan et al. (2012). Physiologically based pharmacokinetic model use in risk assessment--Why being published is not enough. Tox. Sci., 126: 5-15.
40. Meek, ME., Barton, HA, Bessems, JG, Lipscomb, JC, Krishnan K, (2013) Case study illustrating the WHO IPCS guidance on characterization and application of physiologically based pharmacokinetic models in risk assessment, Regulatory Toxicology and Pharmacology, 66 (1), 116-129.
41. Mostrag-Szlichtyng A, Worth A (2010). In silico modelling of microbial and human metabolism: a case study with the fungicide carbendazim. JRC Technical Report EUR 24377 EN.
42. Mumtaz M, Fisher J, Blount B, Ruiz P (2012). Application of Physiologically Based Pharmacokinetic Models in Chemical Risk Assessment. Journal of Toxicology Volume 2012, Article ID 904603, 11 pages.
43. Paini A, Joossens E, Bessems J, Desalegn A, Dorne JL, Gosling JP, Heringa M, Klaric M, Kramer N, Loizou G, Louisse J, Lumen A, Madden J, Patterson E, Duarte Proenca S, Punt A, Setzer WS, Suciu N, Troutman J, Tan YM (2017a). EURL ECVAM Workshop On New Generation of Physiologically-Based Kinetic Models In Risk Assessment. 10.2760/619902.
44. Paini A, Leonard JA, Kliment T, Tan YM, Worth A (2017b). Investigating the state of physiologically based kinetic modelling practices and challenges associated with gaining regulatory acceptance of model applications. Regulatory Toxicology and Pharmacology 90, 104-115.
45. Patel M, Chilton ML, Sartini A, Gibson L, Barber C, Covy-Crump L, Przybylak KR, Cronin MTD, Madden JC (2018). Assessment and reproducibility of quantitative structure-activity relationship models by the non-expert, Journal of Chemical Information and Modelling 58(3):673-682.
46. Patterson EA, Taylor RJ, Bankhead M. (2016). A framework for an integrated nuclear digital environment, Progress in Nuclear Energy 87:97-103.
47. Patterson EA, & Whelan MP. (2017). A framework to establish credibility of computational models in biology, Progress in Biophysics & Molecular Biology, 129:13-19.
48. Pirovano A, Brandmaier S, Huijbregts MAJ, Ragas AMJ, Veltman K, & Hendriks AJ. (2015). The utilisation of structural descriptors to predict metabolic constants of xenobiotics in mammals. Environmental Toxicology and Pharmacology 39(1), 247–258.
49. Price, P. S., Conolly, R. B., Chaisson, C. F., Gross, E. A., Young, J. S., Mathis, E. T., and Tedder, D. R. (2003). Modeling interindividual variation in physiological factors used in PBPK models of humans. Crit. Rev. Toxicol. 33, 469–503.
50. Punt A, Bouwmeester H, Peijnenburg AACM (2017). Non-animal approaches for kinetics in risk evaluations of food chemicals ALTEX 34 , 501-514.
51. Punt A, Bouwmeester H, Schiffelers MJWA, Peijnenburg AACM (2018). Expert opinions on the acceptance of alternative methods in food safety evaluations: Formulating recommendations to increase acceptance of non-animal methods for kinetics. Regulatory Toxicology and Pharmacology, 92, 145-151
52. Rietjens IMCM, Louisse J, Punt A (2011). Tutorial on physiologically based kinetic modeling in molecular nutrition and food research. Mol Nutr Food Res 55:941–956
53. Rotroff DM, Wetmore BA, Dix DJ, Ferguson SS, Clewell HJ, Houck KA, Lecluyse EL, Andersen ME, Judson RS, Smith CM, et al. (2010). Incorporating human dosimetry and exposure into high-throughput in vitro toxicity screening. Toxicol. Sci. 117, 348–358.
54. Saltelli A. What is Sensitivity Analysis? (2000). in Sensitivity Analysis Edited by Saltelli et al., John Wiley & Sons Ltd. Pp. 3-13.
55. SOLVO (2017) Biotechnology, SOLVO The Transporter Book(3rd Edition), Produced and Published by SOLVO Biotechnology(2017)
56. Suciu N, Tediosi A, Ciffroy P, Altenpohl A, Brochot C, Verdonck F, et al. (2016). Potential for MERLIN-Expo, an advanced tool for higher tier exposure assessment, within the EU chemical legislative frameworks. Sci Total Environ, 562, 474-479.
57. Sung JH, Srinivasan B, Esch MB, McLamb WT, Bernabini C, Shuler ML, Hickman JJ (2014). Using physiologically-based pharmacokinetic-guided "body-on-a-chip" systems to predict mammalian response to drug and chemical exposure. Exp Biol Med (Maywood). 239(9):1225-1239.
58. US EPA (U.S. Environmental Protection Agency) (2006). Approaches for the Application of Physiologically Based Pharmacokinetic (PBPK) Models and Supporting Data in Risk Assessment (Final Report). National Center for Environmental Assessment, Washington, DC. EPA/600/R- 05/043F.
59. Teorell T (1937). Kinetics of the distribution of substances administered to the body II. The extravascular mode of administration. Arch Int Pharm Ther 57:205–240
60. Tonnelier A, Coecke S, Zaldívar JM (2012). Screening of chemicals for human bioaccumulative potential with a physiologically based toxicokinetic model. Arch. Toxicol. 86, 393–403.
61. US FDA (U.S. Food and Drug Administration) (2016). Draft “Physiologically Based Pharmacokinetic Analyses — Format and Content Guidance for Industry”. https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM531207.pdf
62. Wagner C, Zhao P, Pan Y, Hsu V, Grillo J, Huang S, Sinha V. (2015). Application of Physiologically Based Pharmacokinetic (PBPK) Modeling to Support Dose Selection: Report of an FDA Public Workshop on PBPK. CPT: Pharmacometrics Syst. Pharmacol., 4(4): 226–230.
63. Wambaugh JF, Wetmore, BA, Pearce R, Strope C, Goldsmith R, Sluka JP, Sedykh A, Tropsha A, Bosgra S, Shah I, Judson R, Thomas RS, Setzer RW (2015). Toxicokinetic triage for environmental chemicals. Toxicol. Sci., 147, 55-67
64. [Waters NJ](https://www.ncbi.nlm.nih.gov/pubmed/?term=Waters%20NJ%5BAuthor%5D&cauthor=true&cauthor_uid=18300299), [Jones R](https://www.ncbi.nlm.nih.gov/pubmed/?term=Jones%20R%5BAuthor%5D&cauthor=true&cauthor_uid=18300299), [Williams G](https://www.ncbi.nlm.nih.gov/pubmed/?term=Williams%20G%5BAuthor%5D&cauthor=true&cauthor_uid=18300299), [Sohal B](https://www.ncbi.nlm.nih.gov/pubmed/?term=Sohal%20B%5BAuthor%5D&cauthor=true&cauthor_uid=18300299). (2008) Validation of a rapid equilibrium dialysis approach for the measurement of plasma protein binding. [J Pharm Sci.](https://www.ncbi.nlm.nih.gov/pubmed/18300299) 2008 Oct;97(10):4586-95. doi: 10.1002/jps.21317.
65. Wetmore BA, Wambaugh JF, Ferguson SS, Sochaski MA, Rotroff DM, Freeman K, Clewell HJ III, Dix DJ, Andersen ME, Houck KA, et al. (2012). Integration of dosimetry, exposure, and high-throughput screening data in chemical toxicity assessment. Toxicol. Sci. 125, 157–174.
66. Wetmore BA, Wambaugh JF, Ferguson SS, Li L, Clewell HJ, Judson RS, Freeman K, Bao W, Sochaski MA, Chu TM, et al. (2013). Relative impact of incorporating pharmacokinetics on predicting in vivo hazard and mode of action from high-throughput in vitro toxicity assays. Toxicol. Sci. 132,327–346.
67. Wetmore BA, Allen B, Clewell HJ, Parker T, Wambaugh JF, Almond LM, Sochaski MA, Thomas RS. (2014). Incorporating population variability and susceptible subpopulations into dosimetry for high-throughput toxicity testing. Toxicol. Sci. 142, 210–224.
68. Wetmore BA (2015). Quantitative in vitro-to-in vivo extrapolation in a high-throughput environment Toxicology 332, 94-101.
69. WHO/IPCS (World Health Organization. International Programme on Chemical Safety). (2010). Characterization and Application of Physiologically Based Pharmacokinetic Models in Risk Assessment. Harmonization Project Document No. 9. Geneva, Switzerland.
70. Worth A, Cronin M, Enoch S, Fioravanzo E, Fuart-Gatnik M, Pavan M, Yang C, (2012). Applicability of the Threshold of Toxicological Concern (TTC) approach to cosmetics – preliminary analysis. JRC report EUR 25162 EN. Publications Office of the European Union, Luxembourg, 2012, available at: [http://publications.jrc.ec.europa.eu/ repository/](http://ihcp.jrc.ec.europa.eu/our_labs/computational_toxicology/doc/ReqNo_JRC68188_LB-NA-25162-EN-N_-PDF.pdf)
71. Yoon M, Campbell JL, Andersen ME, Clewell HJ. (2012). Quantitative in vitro to in vivo extrapolation of cell-based toxicity assay results. Critical reviews in toxicology, 42(8), 633-652.
72. Yoon M, Efremenko A, Blaauboer BJ, Clewell HJ. (2014). Evaluation of simple in vitro to in vivo extrapolation approaches for environmental compounds. Toxicol. in vitro 28, 164–170.
73. Yoon M, Kedderis GL, Yan GZ, Clewell HJ. 3rd. (2015). Use of in vitro data in developing a physiologically based pharmacokinetic model: Carbaryl as a case study. Toxicology 5;332:52-66.
74. Zaldivar Comenges JM, Joossens E, Sala Benito JV, Worth A, Paini A (2017). Theoretical and mathematical foundation of the virtual cell based assay – a review. Toxicol. In Vitro Volume 45, Part 2, Pages 209-221

1. <https://ec.europa.eu/jrc/en/publication/eur-scientific-and-technical-research-reports/eurl-ecvam-strategy> achieving-3rs-impact-assessment-toxicokinetics-and-systemic-toxicity?search [↑](#footnote-ref-1)
2. http://ec.europa.eu/health/scientific\_committees/consumer\_safety/docs/sccs\_o\_190.pdf [↑](#footnote-ref-2)
3. http://www.oecd.org/env/ehs/testing/OECD\_Draft\_GIVIMP\_in\_Human\_Safety\_Assessment.pdf [↑](#footnote-ref-3)
4. Quotation: “The underlining philosophy of read-across is that substances which are similar in chemical structure will have similar properties and thereby, have similar toxicokinetics and toxicodynamics. Experimental derived toxicological proprieties from one substance, often referred to as source chemical, can be read across to fill the data gap for a second substance, the target chemical, which has a similar molecular structure but is lacking data” (Schultz et al., 2015). [↑](#footnote-ref-4)
5. Quotation from Saltelli et al 2000 Sensitivity Analysis – What is Sensitivity Analysis? “For a software engineer, SA could be related to the robustness and reliability of the software with respect to different assumptions “ … “For a statistician, involved in statistical modelling, SA is mostly known and practice under the heading of “robustness analysis” (Saltelli, 2000). [↑](#footnote-ref-5)
6. http://ec.europa.eu/research/participants/portal/desktop/en/opportunities/h2020/topics/sc1-hco-09-2018.html [↑](#footnote-ref-6)