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

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Article

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33

34   **Highlights**

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

36   PBK modelling has the potential to play a significant role in reducing animal testing

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

38   A framework is needed for communicating characteristics and results of PBK modelling

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42

43   **Abstract**

44   The fields of toxicology and chemical risk assessment seek to reduce, and eventually replace, the use of animals

45   for the prediction of toxicity in humans. In this context, physiologically based kinetic (PBK) modelling based on

46   *in vitro* and *in silico* kinetic data has the potential to a play significant role in reducing animal testing, by providing

47   a methodology capable of incorporating *in vitro* human data to facilitate the development of *in vitro* to *in vivo*

48   extrapolation of hazard information. In the present article, we discuss the challenges in: 1) applying PBK

49   modelling to support regulatory decision making under the toxicology and risk-assessment paradigm shift

50   towards animal replacement; 2) constructing PBK models without *in vivo* animal kinetic data, while relying solely

51   on *in vitro* or *in silico* methods for model parameterization; and 3) assessing the validity and credibility of PBK

52   models built largely using non-animal data. The strengths, uncertainties, and limitations of PBK models

53   developed using *in vitro* or *in silico* data are discussed in an effort to establish a higher degree of confidence in

54   the application of such models in a regulatory context. The article summarises the outcome of an expert

55   workshop hosted by the European Commission Joint Research Centre (EC-JRC) - European Union Reference

56   Laboratory for Alternatives to Animal Testing (EURL ECVAM), on "Physiologically-Based Kinetic modelling in risk

57   assessment – reaching a whole new level in regulatory decision-making" held in Ispra, Italy, in November 2016,

58   along with results from an international survey conducted in 2017 and recently reported activities occurring

59   within the PBK modelling field. The discussions presented herein highlight the potential applications of next

60   generation (NG)-PBK modelling, based on new data streams.

61   **Keywords**: Physiologically Based kinetic models; PBPK; PBTK; Toxicokinetics; *In vitro; In silico.-*

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 recognised 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 below (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 characterisation 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).

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", where TK is the abbreviation 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

98   throughout this document we will consistently use the more general terms of PBK model or PBK modelling. It is
99   noted that the ever-increasing advancements *in in* vitro *and in silico* methodologies in the field of toxicology can
100 be used in combination with PBK models to support regulatory decisions on the use of chemical substances. In
101 the present manuscript the term next generation PBK (NG-PBK) model will be used to name these models. This
102 term, NG-PBK, refers to PBK models that are developed without the provision of newly produced (i.e., without
103 animal sacrifice) animal TK data for parametrisation and validation of those models, but rather through
104 supporting *in vitro*, *in silico,* -omics, micro-scale applications. NG-PBK models representing the human body
105 should be parameterized and validated using *in vitro*, *in silico,* -omics data, micro-scale systems, and human *in*
106 *vivo* data, when available. This stands also for PBK models built to represent animals (e.g. livestock, fish, bees),
107 which should be parameterized and validated using *in vitro*, *in silico*, -omics, micro-scale systems and historical
108 or (bio)-monitoring animal data of the species of interest, to avoid the need for animal sacrifice.

109 **Milestones in the history of PBK modelling**

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

123 Over the past 20 years, several workshops have been held to promote the applicability of PBK models in the
124 academic, industrial, and regulatory sectors. For example, a 1995 European Centre for the Validation of
125 Alternative Methods (ECVAM) workshop discussing the use of biokinetic and *in vitro* methods resulted in 15
126 recommendations that were submitted to support and guide future work in the PBK modelling field (Blaauboer
127 et al., 1996). This workshop was followed by many others to better define the potential role of PBK modelling in
128 science and risk assessment following a Three R (replacement, reduction and refinement) strategy (Bouvier
129 d'Yvoire et al., 2007). In the same year, a workshop to address uncertainty and variability analysis in PBK
130 modeling was held by Barton et al. (2007). Loizou et al. (2008) reported the need for clear descriptions of good
131 modelling practices (GMP) for: 1) model development; 2) model characterisation; 3) model documentation; and
132 4) model evaluation. A subsequent thematic workshop aimed to critically appraise PBK modelling software
133 platforms and to provide a more detailed state-of-the-art overview of non-animal based PBK parameterisation
134 tools (Bessems et al., 2014). A CEN (European Committee for Standardization) workshop in 2014 strived for

135 agreement upon the minimum requirements for the amount and type of information to be provided for
136 exposure models, such as PBK models, along with documentation and guidelines for the structure and reporting
137 of such information. The resulting CEN workshop agreement (CWA) was expected to provide a more rigorous
138 means of describing exposure models and to aid users in better understanding them (Ciffroy et al., 2016a;
139 Altenphol et al., 2018). The following year, a workshop assessed the state of knowledge in the application of PBK
140 models in regulatory decision-making, in addition to sharing and discussing best practices in the use of PBK
141 modelling to inform dose selection in specific patient populations (Wagner et al., 2015). In 2017, a workshop
142 organized by the National Centre for the Replacement, Refinement, and Reduction of Animals in Research
143 (NC3Rs) encouraged experts in exposure science to consider the role of PBK models in the extrapolation of
144 external exposure data to internal concentrations to promote the application of non-animal data in efficacy and
145 safety testing (Burden et al., 2017; https://www.nc3rs.org.uk/applying-exposure-science-increase-utility-non-
146 animal-data-efficacy-and-safety-testing). A Lorentz Center workshop entitled "Non-animal Methods for
147 Toxicokinetics: Meeting New Paradigms in Toxicology" was held at the end of 2017 and emphasized the role of
148 PBK models (https://www.lorentzcenter.nl/lc/web/2017/943/info.php3?wsid=943&venue=Oort;
149 https://www.lorentzcenter.nl/lc/web/2017/943/report.pdf). The first European Partnership for Alternative
150 Approaches to Animal Testing (EPAA) partners' forum, held at the end of 2017, aimed to provide an overview
151 on toxicokinetics and read-across with insight into the role of PBK models (Laroche et al., 2018).

152
153 **Framing the problem**

154 The EURL ECVAM Strategy on Toxicokinetics[1], as published in 2015, outlines opportunities for generating and
155 making better use of TK data. The central feature of the strategy focuses on the use of PBK modelling to integrate
156 data from *in vitro* and *in silico* methods for prediction of human whole-body biokinetic behavior, and enables
157 QIVIVE to obtain safety guidance values expressed as external doses (Bell et al., 2018). In the past, *in vivo*
158 tissue/blood concentration-time data were a prerequisite for calibrating and evaluating the predictive capability
159 of a PBK model (Bessems, et al., 2014). The common practice was to start with an animal PBK model, calibrating
160 it with animal *in vivo* data, and then re-parameterizing it based on *in vitro* biotransformation measurements or
161 allometric scaling to develop a human PBK model. As the field of risk assessment evolves towards the goal of
162 reducing, and eventually replacing, the use of animals for predicting human toxicity, PBK model development
163 has seen a shift towards increased use of non-animal data for parameterization, along with increased use of the
164 models for IVIVE. Efforts in this area should be directed towards developing standards that will increase the
165 acceptance of *in vitro* methods for characterizing human-relevant ADME properties. To enhance the acceptance
166 of PBK models at an international level, good modelling practice is required to guide the use of the *in vitro* and
167 *in silico* methodologies in developing PBK models. As the first step, to initiate a dialogue on such a topic, the
168 European Commission's Joint Research Centre (JRC), EURL ECVAM, hosted a workshop on "Physiologically-Based
169 Kinetic modelling in risk assessment – reaching a whole new level in regulatory decision-making" (Ispra, Italy,
170 November 16–17, 2016). The workshop participants discussed challenges in: 1) applying NG-PBK modelling to

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[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

support regulatory decision making; 2) constructing PBK models for safety assessment without animal *in vivo* data, relying solely on *in vitro* or *in silico* methods**;** and 3 assessing the validity 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 per 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). Here, we also discuss recently reported activities in the field, (subsequent to the 2016 EURL ECVAM workshop) that demonstrate both ongoing developments in the field and the continued hesitancy within public health agencies to apply PBK modelling in their decisions. In addition, we will introduce as a new challenge the integration of NG-PBK modelling with toxicodynamic endpoints, as this will be essential for implementation of NG-PBK models.

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 facilitate the successful acceptance of NG-PBK modelling in regulatory decision-making.

1. 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, risk assessors present at the 2016 EURL ECVAM workshop indicated that they prefer to use the simplest model possible, as finding sufficient input data is rather challenging, but would be willing to use more complex models if necessity dictates and sufficient input data are available. 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. Conversely, the regulators can learn what is technically or scientifically feasible and what is not. As such dialogue may prove to be time-consuming, establishing a harmonized template for model construction and evaluation would facilitate the process. The template should be flexible enough for any regulatory agency or country to use, and would ideally incorporate an agreed-upon ontology . To efficiently

206 develop a PBK model to support regulatory risk assessment, modellers and end users (proponents and
207 regulators) need to clearly define their goals of model use and related model requirements at an early stage. For
208 example, if a read-across approach is likely to be applied by the end users, biokinetic data for a pre-determined
209 set of relevant chemicals (target and source chemicals) will constitute important supporting material and should
210 be included in the submission package. In situations where safety assessment is conducted for a new chemical
211 on the market, the following criteria may be used to facilitate regulatory acceptance of a PBK model for this
212 substance: 1) the model should be transparent, with a usable code; 2) model uncertainty should consider
213 biological plausibility, and be clearly described and quantified when possible; 3) uncertainty in exposure
214 scenarios should be characterised, because this uncertainty will propagate to PBK model results; 4) user-friendly
215 platforms should be used where possible; 5) the model should be fit-for-purpose with no unnecessary additional
216 complexity, and with all required parameters measurable; and 6) the model should consider sufficient coverage
217 of chemical space, to allow for read-across approaches if desired. In cases where the model performance needs
218 to be evaluated using human *in vivo* data, regulators may consider using data that are generated from human
219 trials, such as micro-dosing. It should be emphasized that clinical studies would only be conducted once the
220 safety of the chemical has been established and the clinical investigation represents de minimis risk to the
221 subjects.

222

223 **2. Training**

224 Within the current climate of desire to reduce, refine, and replace animal testing through ongoing scientific and
225 technological advancements, it would be beneficial to risk assessors / managers and other workers in safety
226 assessment to be kept abreast of the development of NG-PBK models . In order to achieve this goal, information
227 on a number of novel emerging technologies, in addition to PBK modelling, should be made more accessible.
228 These include -omics, organ- on-a -chip, high-throughput screening methods, read-across, Adverse Outcome
229 Pathways (AOPs), and IVIVE. Additionally, it would be helpful if guidance were available indicating how these
230 different approaches are integrated in support of chemical safety assessment. On the other hand, it is not
231 necessary for risk assessors/managers, etc. to have detailed knowledge related to all the diverse aspects of PBK
232 modelling; rather, it may be sufficient to provide tailored training that focuses only on the specific needs of each
233 regulatory sector and, where applicable, cross-sector needs. For example, some risk assessors may need or wish
234 to run a model, and so they would require knowledge of the relevant software and expertise to review and run
235 model codes. Other risk assessors may rely on a model peer review system to check the implementation and
236 reliability of new model codes, and in this case, may only require sufficient knowledge to allow for interpretation
237 of the data and to enable modelling predictions to be put in context. One option is for risk assessors to assemble
238 technical committees that consist of members possessing a range of expertise, to review the model code and
239 interpret model results. The training content/format should also be tailored to achieve maximum effectiveness
240 in understanding the application of models. In addition to the traditional classroom setting, training formats
241 could include webinars, *ad hoc* short courses, and more refined or specialised graduate-level courses. Further,
242 online training could potentially generate a larger audience that would also allow the modelling and user

243 community to continue to expand. Finally, since risk assessors generally place higher confidence in *in vivo* data,
244 there is a need to make courses on alternative *in vitro* and *in silico* methods more accessible, to provide a path
245 forward to acceptance of these NG-PBK model applications in regulatory decision making.

3. **Guidance**

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

257 The WHO-IPCS published, in 2010, a guidance document on the characterisation and application of PBK models
258 in risk assessment (WHO, 2010). Nevertheless, no comprehensive guidance documentation is currently available
259 for reporting and evaluating NG-PBK models without use of animal *in vivo* TK data, or for interpreting and
260 applying outputs from these models for human safety assessment. Recently, several efforts have been made to
261 produce such documentation. For example, the Scientific Committee for Consumer Safety (SCCS) considers all
262 available scientific data in their safety evaluation of cosmetic substances, including data generated from PBK
263 modelling. In the most recent Notes of Guidance for the Testing of Cosmetic Ingredients and Their Safety
264 Evaluation (SCCS/1564/15)[2], the SCCS defines the conditions for the use of PBK models submitted for risk
265 assessment purposes. PBK modelling has already been accepted as a tool for risk assessment or for use as
266 supporting information in some of the chemical-specific dossiers evaluated by the SCCS, EFSA, and US-EPA. The
267 SCCS document could act as a starting point or as a template for a new general guidance document. Additionally,
268 the new reporting guidelines from the US Food and Drug Administration (FDA) and European Medicine Agency
269 (EMA) (US FDA, 2018; EMA, 2016), on harmonization of reporting and on qualification of PBK modelling and
270 simulation, can also apply to NG-PBK models. To extend this concept, a working group at the Organisation for
271 Economic Co-operation and Development (OECD), comprised of more than 45 scientists from different areas of
272 scientific expertise, are drafting a guidance document for characterizing, validating, and reporting uncertainties
273 in NG-PBK model applications.

274

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

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[2] http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_190.pdf

276 PBK models are built using three sets of parameters: i) physiological and anatomical parameters, with
277 representative reference parameters taken from the species under study (animal or human); ii) biokinetic /
278 ADME properties, which can be gathered using *in vitro* methods or by fitting the model to an in vivo data set;
279 and iii) physico-chemical parameters, which are experimentally derived or obtained using *in silico* approaches
280 such as quantitative activity relationship (QSAR) models (Rietjens et al., 2011). For GMP, the PBK model
281 construction should consider the compound exposure situation/dosing strategy to be simulated (problem
282 formulation). The exposure descriptions should include route of administration, timeframe of the simulation
283 (i.e. exposure duration), and exposure frequency. In the cases of complex models that include inter-individual
284 variability among some physiological values, the number of individuals that should be incorporated into the
285 simulation for sufficient statistical power analysis should also be considered.

286 In the case of NG-PBK models, assuming there is no possibility of generating *in vivo* animal data for the model
287 calibration, there are two key pre-requisites to build the model:

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

291

292 **1. Availability of *in vitro* and *in silico* data for ADME properties**
293 Without *in vivo* data, the values of parameters in a PBK model will need to be derived from the results of *in silico*
294 or *in vitro* experiments. Clearly, the accuracy of PBK models will be heavily reliant upon the quality of the model
295 parameters, which often are not only tissue dependent but also chemical dependent.
296 As it is useful to determine the minimum requirements for PBK models (with respect to data-poor and data-rich
297 chemicals), a decision tree indicating requirements for different scenarios is presented here (Figure 3). The most
298 minimalistic model type, one-compartment models, parameterised with only protein binding and clearance
299 data, have been developed and used to support chemical screening and prioritization (Rotroff et al., 2010;
300 Wetmore et al., 2012, 2013, 2014; Tonnelier et al., 2012; Yoon et al., 2014; Wambaugh et al. 2015). Depending
301 on the exposure route, a compartment representing the skin, intestine, or lung may need to be included in a
302 model. If a compound is highly lipophilic, a fat compartment is required, and it may also be necessary for the
303 model to describe uptake into the lymphatic system. Finally, depending on the hazard data available, additional
304 compartments and biological processes may need to be added to the PBK model. Throughout development of
305 the model, as more specific information is obtained on the chemical's properties and mode of action (MoA),
306 confidence is increased in the applicability of the models. A good strategy would be to begin with a generic
307 model structure, then move to more specific models once knowledge is gained that indicates a unique biokinetic
308 behavior of the compound in question. In using a simple model, it is possible that a key kinetic pathway specific
309 to a given target chemical will not be taken into consideration. To address this issue, a database of all known
310 ADME/TK processes, such as cell uptake (capturing the role of transporters), metabolism, and efflux, could be
311 developed to help modellers identify which processes may need to be included for a specific chemical / purpose.
312

313 Membrane transporters influence the ADME processes of various endogenous and exogenous compounds
314 (Klaassen and Aleksunes, 2010; SOLVO, 2017). In recent decades, the pharmaceutical field has placed
315 considerable effort into the study of transporters affecting drug disposition, therapeutic efficacy, and adverse
316 outcomes, but little is known in regards to transporter effects on environmental chemicals (Clerbaux et al.,
317 2018). Transporters can play a significant role in chemical distribution. As such, integration of membrane
318 transporter-based experimental data during parameterization of several types of computational models (e.g.,
319 QSAR, pharmacophore, and PBK models), through use of platforms like SimCyp, PKSim, or GastroPlus, will
320 enable better understanding of chemical/drug disposition (Clerbaux et al., 2018).

321

322 Protein binding in plasma influences the partitioning of endogenous and exogenous compounds from the blood
323 into the tissues. The plasma protein binding property is, among other things, related to lipophilicity, as binding
324 becomes greater with more lipophilic chemicals, thus sequestering such chemicals in blood and limiting the
325 systemic availability and distribution of unbound fraction of the chemical. A common and widely used method
326 for estimating plasma protein binding *in vitro* is the rapid equilibrium method, which involves measurement of
327 chemical transport across a dialysis membrane with a high surface area-to-volume ration within a Teflon-lined
328 plate well (Waters et al., 2008).

329

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

342

343 *In vitro* data for metabolism may be generated using tissue slices, organ (e.g., liver) homogenates, cell lines,
344 spheroids, or (sub)cellular fractions (such as microsomes, baculosomes, S9, and cytosol1), where metabolism is
345 measured as loss of the parent compound or production of metabolite(s). It should be noted that if metabolism
346 occurs very slowly, it may not be detected in a short-term *in vitro* assay. If a chemical is known to be
347 predominantly excreted unchanged in urine, then metabolism is less relevant to the model. However, if
348 metabolism of a parent compound is thought to be metabolized to undergo biliary excretion or to be excreted
349 via the bile, then a model including such elimination pathways is necessary, first by determining which pathways
350 of elimination are most relevant to the target chemical. *In silico* and *in vitro* models have also been developed

351 for predicting different processes involved in elimination. These include *in silico* models for total clearance

352 (Lombardo et al., 2014) and metabolism (Pirovano et al., 2015) and *in vitro* models for biliary excretion (Ghibellini

353 et al., 2006). However, more work is required to develop models for elimination, and the applicability domain

354 for existing models should be carefully considered before application to a wider range of chemicals. A current

355 limitation is that there are no (OECD) guideline(s) addressing *in vitro* methods to determine kinetic parameters,

356 except for the guideline on Skin Absorption (OECD TG 428). In the absence of standardised methods for

357 generating *in vitro* parameters to calibrate PBK models, it is important that *in vitro* metabolism data or data

358 regarding transporters are produced according to the new OECD good *in vitro* method practice (GIVIMP)[3]. The

359 GIVIMP document is meant to serve as technical guidance on generating and applying quality data through good

360 scientific and quality practices, to support the regulatory human safety assessment of chemicals using *in vitro*

361 methods.

362

363 Bessems et al. (2014) provides a general overview of the currently available *in vitro* and *in silico* methods for

364 characterizing human ADME and the gaps and challenges faced. Mostrag-Szlichtyng et al (2010) provide an

365 extensive review specifically of *in silico* tools (i.e., QSAR models and software) for prediction of ADME properties

366 that are relevant to PBK model building. More recently, Patel et al (2018) have collated and assessed the quality

367 of over 80 models for 31 absorption-, distribution-, and excretion-related endpoints (Patel et al., 2018).

368

369 Finally, toxicodynamic data derived from *in vitro* toxicity tests are typically based on nominal concentrations of

370 the substances, which may contain significant errors due to the loss of biological, physical, and toxicological

371 chemical processes in such tests. An *in vitro* biokinetic study plays a significant role in translating a nominal

372 concentration used in *in vitro* systems to the actual level of cell exposure producing the effect. Several

373 methodologies can be applied to address such a relationship, such as *in vitro* fate and transport mass balance

374 models recently developed by several research teams (Kramer 2010a, 2010b; Armitage et al., 2014; Fischer et

375 al., 2017; Zaldivar Comenges et al., 2017).

376

377   **2. Availability of modelling platforms**

378 Currently, several open source modelling platforms, such as IndusChemFAte (Cefic LRI, http://cefic-

379 lri.org/toolbox/induschemfate/), High-Throughput Toxicokinetics (httk)-r package (Wambaugh et al., 2018,

380 https://cran.r-project.org/web/packages/httk/index.html),   MEGEN-RVis   (Loizou   et   al,   2011;

381 https://megen.useconnect.co.uk/),   PLETHEM   (http://www.scitovation.com/plethem.html),   MERLIN-EXPO

382 (Ciffroy et al., 2016b; Suciu et al., 2016; https://merlin-expo.eu/), and PK-Sim (www.systems-biology.com), and

383 license-based   platforms   such   as   GastroPlus   (www.simulations-plus.com)   and   SimCyp

384 (https://www.certara.com), are available to individuals possessing varying degrees of expertise in PBK

385 modeling. These platforms provide different computational tools that allow non-programmers to develop and

386 run model simulations with varying options to gain a better understanding of model behavior, which is essential

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[3] http://www.oecd.org/env/ehs/testing/OECD_Draft_GIVIMP_in_Human_Safety_Assessment.pdf

387 for interpretation of model output. The PBK models run from these platforms can be parameterised using *in*

388 *vitro* or *in silico* data. However, programmers or users with modeling skills can also use R, MATLAB, and Berkeley

389 Madonna software to develop customised PBK models, and to support the generation of innovative modeling

390 components, which might otherwise not be generated through use of the more-structured commercial

391 platforms.

392

393 A concern for the use of open source modelling platforms, as compared to use of their proprietary counterparts,

394 is the lack of sustainable resources and funding that are needed for further development and maintenance of

395 those platforms. While most of these platforms are initiated by a research grant, upon completion of the project,

396 the developers are often unable to find other funding sources to maintain it. In order for a modelling platform

397 to remain sustainable, it is essential to maintain access to the model's equations, so that these can be easily

398 coded later. Sustainability also depends on the ability of model updates to be communicated to end-users.

399 Establishment of an open source library as a repository for all available model information, including a peer

400 review process, is strongly recommended.

401

402

403 **3. Integrating NG-PBK modelling with toxicodynamic endpoints**

404 There is high value in the use of PBK models to predict internal target tissue doses for risk assessment

405 applications, based on the assumption that a similar tissue response arises from an equivalent target tissue dose,

406 rather than the external dose, across different exposure conditions. In addition, toxicodynamic processes that

407 that are interpreted in a high-throughput context from *in vitro* dose–response data can be integrated with PBK

408 models, to link external exposure concentrations to target tissue doses to adverse endpoints. Such integration

409 allows for support of several risk assessment extrapolations, such as QIVIVE and reverse dosimetry approaches.

410 Examples of PBK/TD models are reported in table 3 of Punt et al., 2011. However, the application of PBK/TD

411 models in risk assessment requires proper evaluation of model purpose, model assumptions and structure,

412 mathematical representation, parameter estimation, computer implementation, and predictive capacity.

413 The topic of model evaluation will be captured in the next chapter.

414

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

416 A question that often arises is "*How can we trust a PBK model prediction if there are no in vivo data to evaluate*

417 *the simulation; how can the model gain credibility then?*"

418 The following approaches could be applied and are described in further detail below: 1) read-across; 2) micro-

419 scale systems; 3) pragmatic conservative scenario approach; 4) "credibility matrix"; 5) the reliability of dose

420 metric predictions provided with uncertainty and sensitivity analyses (WHO 2010); and 6) population

421 characteristics and virtual population libraries.

422

423   **1. Read-across**

424 For those cases in which *in vivo* data exist for one chemical, a read-across approach[4] may be applied to

425 parameterize models for other chemicals (Schultz et al., 2015). For example, if a valid PBK model exists for

426 chemical A (source chemical), and chemical B (target chemical) lacks any *in vivo* data and has been shown to be

427 similar in structure to chemical A, then the same parameterised PBK model structure/code and *in vivo* data for

428 chemical A can be used for chemical B. This read-across approach has been demonstrated by case studies

429 applying the PBK Knowledgebase developed by Lu et al. (2016). Alternatively, if parameterisation of the PBK

430 model using available *in vitro* or *in silico* data for chemical B is possible, predictions can be compared to output

431 from the model for chemical A based on *in vivo* data, in order to evaluate the PBK model for chemical B. When

432 using such a model based on similarity between different chemicals, the influence of chemical-specific

433 properties mediating ADME behaviour (e.g., log P, specific functional groups) should be carefully considered.

434

435   **2. Micro-scale systems**

436 Microscale systems, such as human-on-a-chip technology, could potentially be applied to measure and predict

437 kinetics and whole body response to substances (Sung et al., 2014), thus aiding in evaluation and increased

438 confidence in NG-PBK models. However, the limitations of these novel microscale systems should be carefully

439 considered. For example, flow rates from model systems are often not scaled down in a similar manner as tissue

440 volumes, thus rendering interpretation of the data difficult for PBK model applications.

441

442   **3. Pragmatic conservative scenario approach**

443 When *in vivo* data are lacking for model evaluation, a pragmatic conservative scenario could be followed in order

444 to derive the most conservative estimate for risk assessment. For NG-PBK modeling, such an approach needs to

445 be designed in such a way that the structure and input of the model is likely to lead to an overestimation of the

446 internal concentration. This can be achieved by including uncertainty factors in the input parameters of the

447 model. A worst-case estimate for absorption can for example be set to 100%. Other input parameters, such as

448 metabolic clearance can be set to a value that is a certain extent lower than that measured for *in vitro* rates. To

449 define the conservative boundaries around each input parameter, the uncertainties of each *in vitro* or *in silico*

450 input method need to be identified.

451

452   **4. Credibility matrix**

453 There is a need to develop a framework for supporting the credibility of PBK models in support of risk assessment

454 applications. As a first requirement for credibility, PBK models should be biologically plausible. Often, modellers

455 or mathematicians exclude a number of biologically-relevant processes because these processes are considered

456 to have no bearing on the model results and because models should be kept as simple as possible created

---

[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).

457 following the required purpose/problem formulation. However, such assumptions must always be discussed and

458 agreed upon with biologists and toxicologists, to prevent the omission of critical biological and toxicological

459 steps or key events. Good documentation of model assumptions is critical for modelers to demonstrate the

460 validity of their models to reviewers and users, and visualization is a key feature when dealing with

461 communication of these models. The recent EFSA uncertainty guidance document provides a reporting table for

462 listing and evaluating model uncertainties (EFSA 2018).

463

464 From the 2016 EURL ECVAM workshop, the following graphical representation and application of the "credibility

465 matrix by Patterson & Whelan" has been proposed. The matrix (Figure 4) allows for locating a specific model

466 type based on the information available, i.e whether a model is principled and testable, as well as knowledge of

467 the biology and the availability of data, which should aid in systematically establishing model credibility via a

468 process of social epistemology (Patterson & Whelan, 2017). If a model falls in the bottom left region (testable

469 and with full knowledge), confidence in the model is likely high. However, if a model falls in the top right region

470 of the matrix (not testable and without any knowledge of the system biology), confidence in the model is likely

471 low due to the uncertainties associated with it. In other words, regulators are unlikely to trust model types found

472 in the top right region of the matrix when making decisions. The question is, to what degree a PBK model would

473 need to be placed towards the bottom left corner to attain sufficient credibility for regulators. In some sense,

474 testable models do not really predict, but provide an estimate to compare against available data in a

475 retrospective fashion.

476

477 The proposed framework should lay out the requirements for validating models with different degrees of

478 knowledge and testability (e.g., quantitative validation), which could aid in quantifying the uncertainty currently

479 existing with animal models, and which can help regulators assess whether models developed through *in vitro*

480 and *in silico* methodologies can be equally reliable, or even more so, compared to current risk assessment

481 approaches. Biological systems, by nature, are complex networks operating under simple rules that can be

482 described by non-linear dynamic processes, and which exhibit non-trivial emergent and self-organizing behavior.

483 As a result, a measured value might represent a particular, and perhaps unknown, state of a system, which

484 makes its use, as a comparator for a predicted value, challenging. To handle such issues, approaches that

485 operate on experience-based validation are required. Ideally, these, approaches would capture the diversity of

486 experiences to establish generic digital twins, which are couplings of validated models with their real-world

487 datasets (see Patterson et al., 2016).

488

489 There is disagreement amongst modellers as to the meaning of the terms model evaluation, verification, and

490 validation; for instance, EMA has shifted to use of the word "qualification". Regardless of which term is more

491 appropriate, the analytical purpose is to ensure that the model is appropriate for the task at hand, and that its

492 predictions are a reasonable representation of reality. Once confirming that the model is a reasonable

493 representation of reality for the intended purpose, several analyses may be used to "validate" a model, including

494 sensitivity analysis, robustness analysis[5], assumption justification, model argumentation, structured calibration,

495 predictive performance, proper scoring rules, and relation to reality. To "verify" a model, the model scope should

496 be revisited and the model equations and code reviewed. The following key elements were suggested by the

497 2016 EURL ECVAM workshop participants to achieve model credibility (Paini et al., 2017a):

498   ✓ Understand the model;

499   ✓ Understand the data underpinning the model;

500   ✓ State clearly the assumptions and hypothesis encoded;

501   ✓ Consider the gap between the model and reality, based on available observations.

502 This last item can be a description of what is lacking in the model. The outcomes of sensitivity analyses can be

503 used to explain some model deficits. One possible approach, as opposed to the statement in the introduction

504 regarding developing the simplest model, would be to start with a more complex model and then remove

505 parameters to which the predictions are not sensitive. The potential problem with this approach is that when

506 there are many parameters with large uncertainties, they may introduce a great deal of variation into the

507 uncertainty analysis.

508

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

510 In 2010, the World Health Organization (WHO) reported the level of confidence needed to gain credibility in a

511 PBK model intended for risk assessment (WHO, 2010). The degree of confidence in a PBK model's predictions

512 depends upon how well the model has been tested against real data and whether adequate sensitivity and

513 uncertainty analyses have been conducted, in order to support the reliability of predictions (WHO, 2010). In the

514 case of NG-PBK models, the lack of "real data" (e.g *in vivo* human data) that are required to evaluate model

515 predictions for the purpose of validation render such validation nearly impossible. However, reporting of

516 adequate sensitivity and uncertainty is certainly relevant and encouraged. Tables providing guidance in

517 reporting results of uncertainty and sensitivity analyses have been provided in the WHO 2010 article, as a tool

518 to better document the evaluation of model predictions (from WHO 2010; Meek et al. 2013). There are several

519 areas that are considered to present current challenges in accepting model-informed drug development, which

520 can also provide insight into necessary acceptance criteria for PBK model-based drug development. Among

521 those criteria, most noteworthy is that the adequacy of submitted PBK models is to be based on their intended

522 purposes at different stages of drug development (Paini et al., 2017a). That is, determination of whether a model

523 is fit-for-purpose and the need to identify and transparently communicate the knowledge gaps. EMA and US

524 FDA published a draft document in 2016 as guidance on the qualification and reporting of physiologically based

525 pharmacokinetic (PBPK) modelling and simulations (EMA, 2016; US FDA 2018). The aim of this guideline is to

526 describe the expected content that should be included in PBK modelling and simulation reports during regulatory

527 submission, including applications for authorization of medicinal products, pediatric investigation plans, and

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[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).

528   clinical trial applications. This also includes the documentation needed to support the qualification of a PBK

529   platform for an intended use, such as results of sensitivity and uncertainty analyses.

530   **6.   Population characteristics and virtual population libraries**

531   This chapter reports information on population characteristics as virtual population libraries for the *in silico*

532   medicine field. However we believe that this information could be also relevant for the chemical risk assessment.

533   Efforts undertaken to better capture the heterogeneity in the human species can certainly be applied to

534   environmental chemical risks, as different population cohorts may be more at risk to specific chemical exposures

535   than are other cohorts. Important aspects of human heterogeneity include inter-individual variations in lifestyle,

536   health status (immunosuppressed, disease patient) genetic polymorphism (gene expression), physiology (uptake

537   rate), biochemistry and molecular biology (Mclanahan et al., 2012), all with respect to age. These factors will

538   interact and influence the chemical ADME and biokinetic behaviors and toxicodynamics within the body.

539   Parameters in a PBK model have a direct biological correspondence, providing a useful framework for

540   determining the impact of observed variations in physiological and biochemical factors on the population

541   variability in the achieved target of a particular chemical (Clewell and Andersen, 1996; Price et al., 2003;

542   Mclanahan et al., 2012). In addition, integration of genetic information from –omics studies will enhance

543   predictions for precise and personalized medicine. Applications for predicting the kinetics of substances within

544   specific populations, such as in the field of pediatrics, have been increasing in their development and use (Leong

545   et al., 2012). In the pharmaceutical field, population-specific PBK models can simulate untestable clinical

546   outcomes, allowing for evaluating the effects of intrinsic (e.g., organ dysfunction, age, genetics, etc.) and

547   extrinsic (e.g., drug-drug interactions) factors, alone or in combination, on drug target concentrations.

548

Next steps and future perspectives

With an increasing demand for application of alternative methods within the risk assessment framework, the need for the 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, 2018) 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 in making appropriate use of *in vitro* data and/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 are 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 *in vitro* and *in silico* methodologies to estimate 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 standardised 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 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 threshold of toxicological concern (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

585 PBK models that have been built and/or peer reviewed, and once this repository is developed, relevant
586 documentation can be introduced from an independent peer review to support model credibility. Such a
587 repository is in line with the work reported in Lu et al., (2016) and will allow for the curation of more case studies
588 and the creation of libraries of *ad hoc* PBK models that could be used for training purposes. Additionally, this
589 repository will facilitate risk assessment approaches applying PBK models and IVIVE, and communicate to
590 decision makers more efficiently the current state of science regarding the use of animal-free models in
591 regulatory applications. Perspectives from the various industrial stakeholders (e.g. pharmaceutical, food safety,
592 agricultural, and personal care product industries) also need to be communicated, to provide greater insight of
593 current practice and understanding of future needs of these sectors, to enable promotion of best practices.

594 Application of NG-PBK models, in the context of exposure in specific population of patients, would be extremely
595 valuable in the generation of virtual population/patient libraries. These libraries would enable clinical trials to
596 entail populations with a greater number of "virtual" individuals, which might not otherwise be possible to
597 conduct with a limited number of real persons/patients. Additionally, these libraries would introduce
598 populations more rarely encountered, such as those possessing enzyme polymorphisms that exert a greater
599 influence on drug-drug interactions or those with rare genetic diseases or health abnormalities. Such libraries
600 would also prove useful in chemical risk assessment when evaluating interindividual variability in relation to
601 chemical exposures and toxicological outcomes.

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

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

609 i) development of more transparent, accessible, and user-friendly software platforms that facilitate
610 development and application of PBK models by a community of users, and which allow specific populations to
611 be modelled or population variability to be evaluated;

612 ii) development of resources to inform new developments in *in silico* and *in vitro* approaches that may be used
613 to provide data for model development;

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

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

619   v) GMPs and harmonised guidelines for reporting the steps taken during model development, evaluation, and

620   application, with respect to NG- PBK models. This would include the use of a clear and common terminologies.

621

622

632   **Disclaimer**

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841

Figure Legend

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

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

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

851 **Figure 4.** Credibility matrix showing comparative loci for a model based on traditional *in vivo* data-based approaches and for
852 a model based on an alternative approach (i.e., *in vitro*, *in silico* methods and/or micro-scale systems). The rationale for the
853 locations of the model types, indicated by stars and letters, are given in the side-bar legend. For example, *in silico* models
854 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
855 to a more sophisticated, but still heuristic, model 'b' based on the understanding gained from model 'a'. The predictions
856 from models 'a' and 'b' are used to design *in vitro* tests that enable the development of model 'c', which can be validated
857 using the rational-empirical approach, thus enhancing its credibility. Finally, this leads to the development of clinical studies
858 and model 'd', supported by its predecessors and quantitatively validated or confirmed using clinical data. This places model
859 'd' in the bottom left corner, as a model whose predictions stakeholders, including regulators, practitioners, and patients,
860 will likely use to make decisions (adapted from Paini et al., 2017a, proposed by Patterson and Whelan 2017).
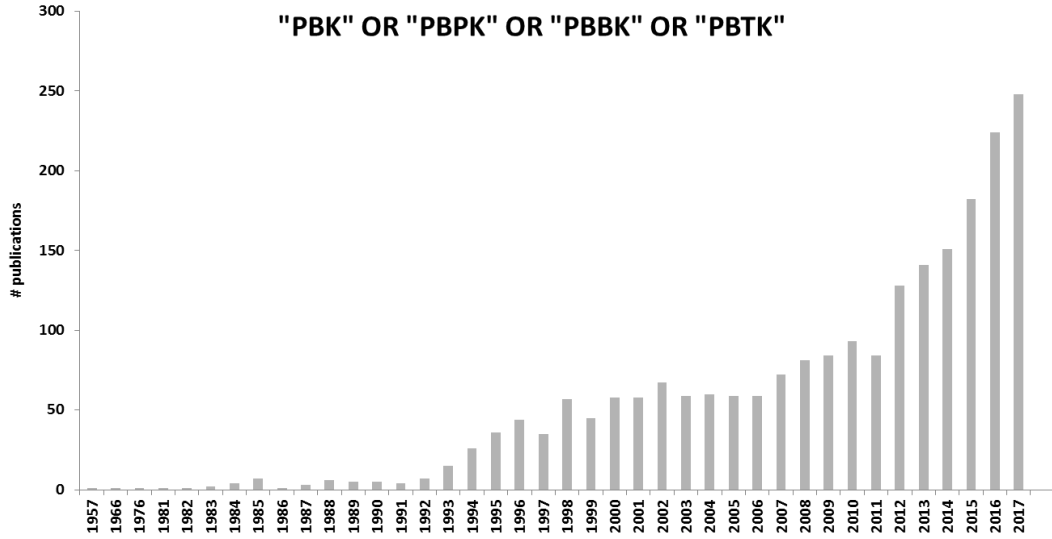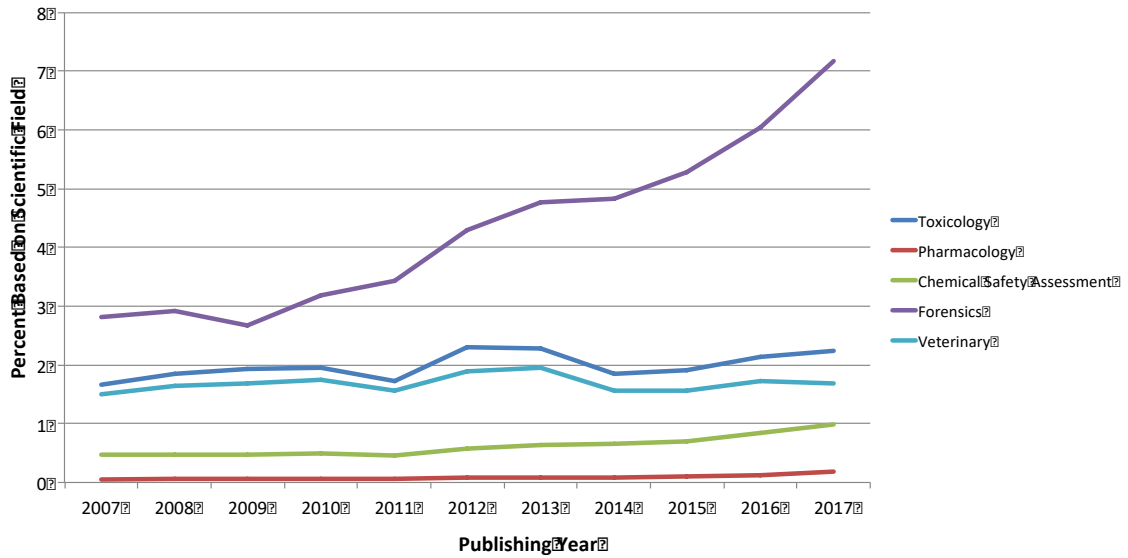861

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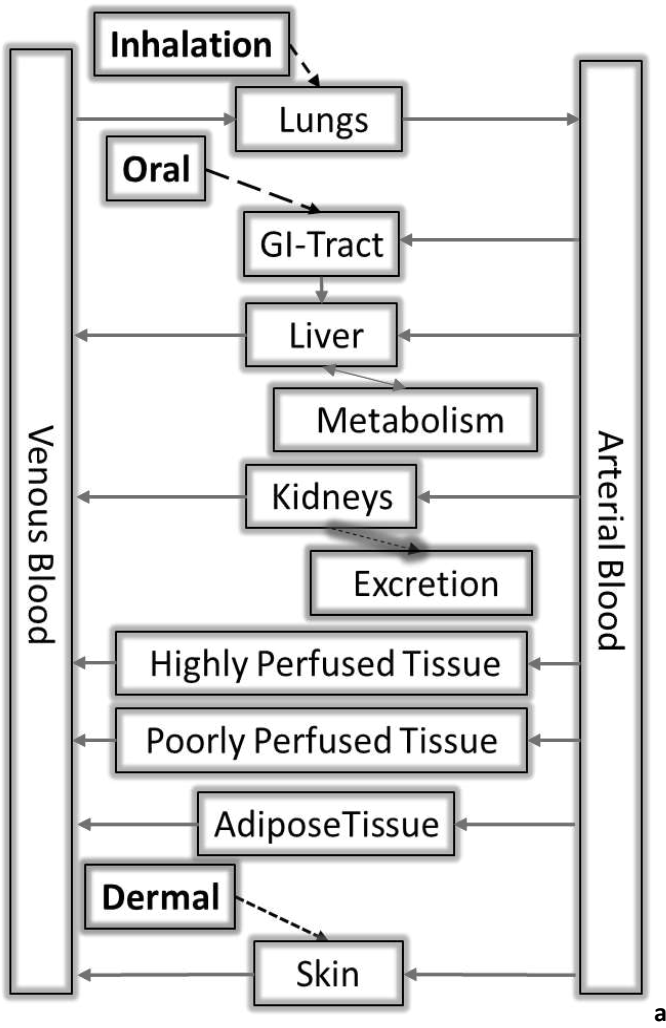
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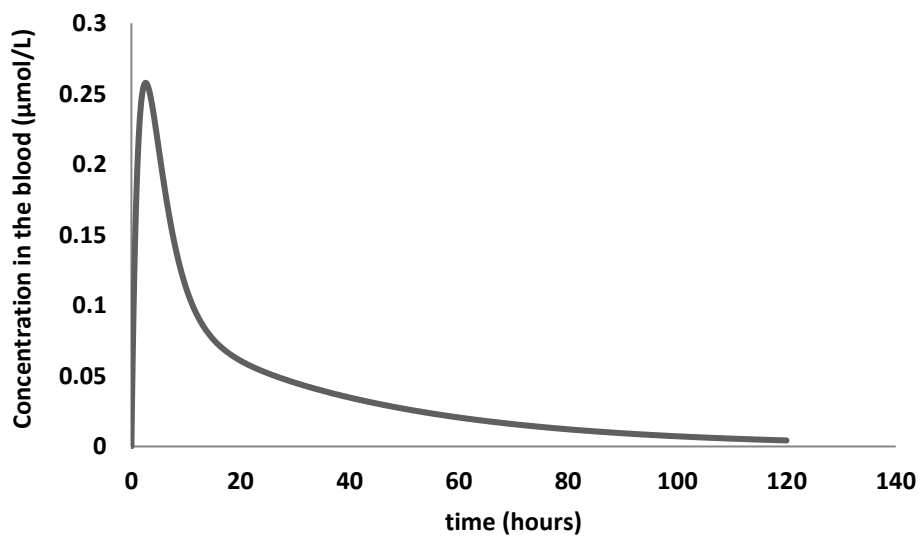
864

865   Figure 1. Paini et al.,

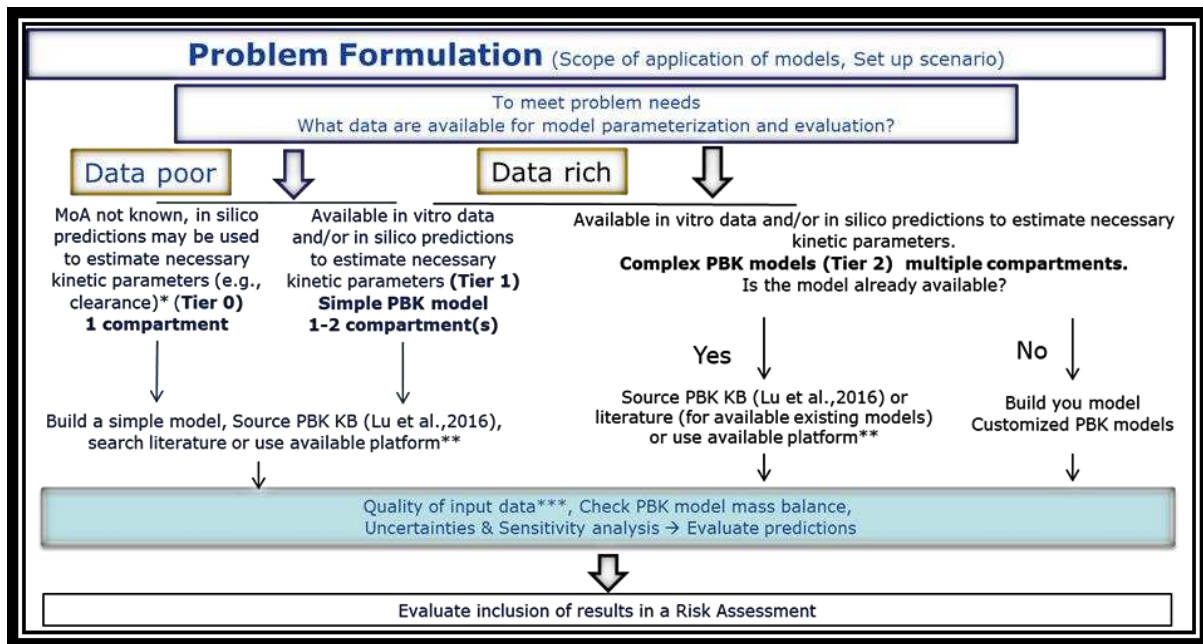866

"PBK" OR "PBPK" OR "PBBK" OR "PBTK"

A.

B.

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869   Figure 2 Paini et al.,

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MoA = Mode of Action; WF = workflow; PBK = Physiologically based kinetic; KB = Knowledge Base
*For instance predictions of input parameter estimated using QSARs
** For instance R/Httk, Plethem, SimCyp, Gastroplus, Pksim, IndusChemFate among others

***QUALITY OF INPUT DATA
In vitro:
a. Capture in vitro artefacts
b. If available follow OECD TG or Guidance Document on Good In Vitro Method Practices (GIVIMP)
c. Report results applying OECD harmonized templates
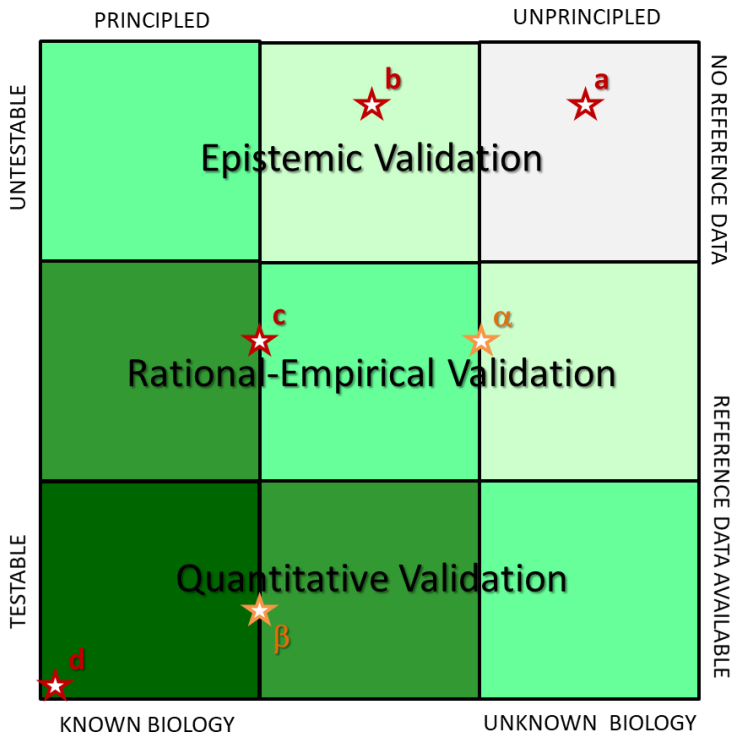
In silico (applicable to QSARs):
follow the OECD 5 principles for validation:
a. a defined endpoint
b. an unambiguous algorithm
c. a defined domain of applicability
d. appropriate measures of goodness-of–fit, robustness and predictivity
e. a mechanistic interpretation, if possible

Human/animal (bio)monitoring data

871

872   Figure 3. Paini et al

Alternative approach

(a) *in silico* model based on untested hypothesis with no reference data
(b) Heuristic model based on understanding gained from (a);
(c) Predictive model based on understanding from (b) & supported by well-designed (using heuristic model) *in vitro* tests;
(d) Predictive model based on understanding gained from (c) & supported by human data and will be used for decision-making.

Traditional approach

(α) Predictive model based on & supported by in vitro tests using hypothesis driven experiment design;
(β) Predictive model based on understanding from (α) & supported by animal data from *in vivo* tests and will be used for decision-making.

NOTE:
Exact position of model in each box depends on the case being considered; as does allocation to a box when model lies on a boundary.

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875   Figure 4. Paini et al.

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