Guidance document on the characterisation, validation and reporting of Physiologically Based Kinetic (PBK) models for regulatory purposes

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A cooperative agreement among **FAO, ILO, UNDP, UNEP, UNIDO, UNITAR, WHO, World Bank and OECD**

Foreword

OECD Member Countries have been making efforts to expand the use of alternative (non-animal) methods to aid the chemical risk assessment process. In this context, the OECD has developed this guidance document on Physiologically Based Kinetic (PBK) models, with the goal of increasing confidence in the use of these models parameterised with data derived from *in vitro* and *in silico* methods. The document provides insights into how the data generated by such methods can be applied to construct PBK models and how these models can be validated. The use of scientifically valid PBK models will allow chemical assessment to rely on the use of these approaches for toxicity testing, rather than *in vivo* data derived from animal studies. A series of cases studies illustrate the use of PBK models based on *in vitro* and *in silico* data, along with the application of the model assessment framework proposed herein.

While the guidance provides contextual information on the scientific process of PBK model characterisation and validation, it is not intended to provide technical guidance on PBK model development or best practices for modellers. Similarly, while case studies are provided to illustrate possible applications of PBK models, guidance on how to use PBK models for specific regulatory purposes is out of scope. It is noted that the level of confidence required for a PBK model will be dependent on the regulatory context of use, and is determined by the regulatory assessor. The primary goal for this guidance document is to provide a clear and consistent model assessment framework for facilitating the dialogue between the developers and proponents of PBK models and regulators who review and adopt the use of PBK models.

The development of this document was led by the European Commission (Joint Research Centre) and the US Environmental Protection Agency. An initial draft was developed with contribution of the OECD PBK Expert Group, reviewed by the Working Party on Hazard Assessment (WPHA) and Extended Advisory Group on Molecular Screening and Toxicogenomics (EGMST) and endorsed by WPHA. This report is published under the responsibility of the OECD Chemicals and Biotechnology Committee.

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List of abbreviations

Abbreviation	Explanation
ACAT	Advanced compartmental absorption and transit
ADAM	Advanced dissolution, absorption and metabolism
ADME	Absorption, distribution, metabolism and excretion
AOP	Adverse Outcome Pathway
AUC	Area Under the (concentration-time) Curve
BBB	Blood Brain Barrier
BCF	Bioconcentration Factor
BDDCS	Biopharmaceutical Drug Disposition Classification System
CEN	European Committee for Standardization
CL	Clearance
Cl_{int}	Intrinsic Clearance
C_{max}	Maximal peak concentration
C_p	Plasma concentration
EC; EC_{50}	Effective Concentration
ECCS	Extended Clearance Classification System
eFAST	Extended Fourier Amplitude Sensitivity Test
EFSA	European Food Safety Authority
EMA	European Medicines Agency

Abbreviation	Explanation
EPA	US Environmental Protection Agency
FDA	US Food and Drug Administration
FIM	Fisher Information Matrix
GFR	Glomerular Filtration Rate
GIVIMP	Good *in vitro* Method Practices
GSA	Global sensitivity analysis
I.V.	Intra venous
IC; $IC5_0$	Inhibitor concentration
IPCS	International Programme of Chemical Safety
ISEF	Inter-system extrapolation factor
J_{max}	Maximum pathway flux
K	Rate constant
K_{ba}	Solid-phase microextraction
K_{el}	Elimination rate constant
K_m	Concentration at half-maximal rate
K_m	Substrate Concentration, Michaelis constant
K_{oa}	Octanol-air partition
K_{ow}	Octanol-water partition
LH	Latin Hypercube
LOD	Limit of Detection
Log D	Distribution coefficient
Log P	Repartition coefficient
MoA	Mode of Action
MoE	Margin of exposure

Abbreviation	Explanation
MoIE	Margin of internal exposure
MW	Molecular weight
OAT	One-at-a-time
OECD	Organisation for Economic Cooperation and Development
OECD TG	OECD Test guideline
PAMPA	Parallel artificial membrane permeability assay
Papp	Apparent permeability coefficient
PBK	Physiologically Based Kinetic (Model)
PBPK	Physiologically Based Pharmacokinetic (Model)
PK	Pharmacokinetics
pKa	Dissociation constant
PoD	Point of Departure
Q-IVIVE	Quantitative - *in vitro* to *in vivo* extrapolation
QSAR	Quantitative structure-activity relationship
RAF	Relative activity factor
RT-HEP	Rainbow trout – hepatocyte
SA	Sensitivity analysis
t½	Half-life
TK	Toxicokinetics
T_{max}	Time of maximum drug concentration, peak time
UA	Uncertainty analysis
VCBA	Virtual Cell Based Assay
Vd	Volume of distribution

Abbreviation	Explanation
VIVD	Virtual *in vitro* distribution model
V_{max}	Maximal rate
WHO	World Health Organization

Chapter 1. Introduction and scope

1.1. Purpose and scope

The aim of this document is to provide guidance on the characterisation and reporting of Physiologically Based Kinetic (PBK)[1] models used in the regulatory assessment of chemicals. Emphasis is placed on evolving applications in which the assessment relies on the use of *in vitro* and *in silico* (non-animal) approaches for toxicity testing, rather than *in vivo* data derived from animal studies. Compared with traditional applications based on the use of *in vivo* data, the new paradigm of toxicity testing introduces "unfamiliar" uncertainties related to the confidence placed in the methods, and how the data generated by such methods can be extrapolated to the *in vivo* level by means of a PBK model. In this case, the inputs to a PBK model are also based entirely on non-animal data and no *in vivo* kinetic data are available to support model calibration and/or validation. To address this particular challenge, an assessment framework for PBK models is needed to capture the attributes and uncertainties of PBK models developed for evolving applications, including "data-poor"[2] situations.

This guidance is expected to promote the use of PBK models in regulatory risk assessment by providing a harmonised framework to facilitate dialogue between the main actors, namely the developer of a PBK model, the proponent of the model in a regulatory submission, and the regulator who assesses the applicability of the model in a given decision making context.

While the guidance provides contextual information on the scientific process of PBK model characterisation and validation, it is not intended to provide technical guidance on PBK model development for modellers. Similarly, while case studies are provided to illustrate possible applications of PBK models, guidance on how to use PBK models for specific regulatory purposes is out of scope. It is noted that the level of confidence required for a PBK model will be dependent on the regulatory context of use, and is determined by the regulatory assessor.

This guidance is applicable to PBK models for chemicals used in a range of products, except for medical devices and products where guidance is already established (see Section 1.4 below). In principle, this guidance is applicable to chemicals in the nanoform (nanomaterials; NMs), biologicals, macromolecules, and metals but would need to be extended to capture the additional uncertainties relating to the kinetics and mode of action (MoA) of these compounds, and the status of non-animal methods to characterise the models.

This guidance is applicable to PBK models developed for humans (in different life stages and vulnerable groups), laboratory test species (e.g. rats, mice, dogs and rabbits), farm animals and species of ecological relevance (birds, fish etc.).

This version of the guidance is based on the current state-of-the-art in the area of PBK modelling. It is foreseen that an update may be required in the future as more experience is gained and new technologies, evidence and applications emerge.

1.2. Specific aims

The specific aims of this guidance document are to:

1. Summarise a scientific workflow for characterising and validating PBK models, with emphasis on models that are constructed using *in vitro* and *in silico* data for absorption, distribution, metabolism and excretion (ADME) parameters.
2. Identify knowledge sources on *in vitro* and *in silico* methods that can be used to generate ADME parameters for PBK models.
3. Develop an assessment framework for evaluating PBK models for intended purposes, with emphasis on the major uncertainties underlying the model input data, structure and predictions.
4. Provide a template for documenting PBK models in a systematic manner.
5. Provide a checklist to support the evaluation of PBK model applicability according to context of use.

1.3. Basics of PBK modelling

PBK models are a mathematical representation (based on ordinary differential equations) of biological processes in the body. They allow the prediction of absorption, distribution, metabolism and excretion (ADME) of a chemical in humans and other animal species. The current guidance does not go deep into the methodological details of the modelling approach or the "non-animal" methods used to parameterise the model, but assume the readers have some understanding of these basic concepts.

A glossary of terms is provided at the end of this document. In addition, Table 1.1 provides references to selected introductory materials (not comprehensive) to aid the non-expert to get acquainted with the PBK modelling approach and its use in chemical risk assessment.

Table 1.1. Selected introductory literature and resources on PBK model development and applications.

Topic	Reference
Tutorial on how to build PBK models	Kuepfer et al., 2016 Rietjens et al., 2011, Andersen 2003, Campbell et al., 2012 among others. In addition available web-based presentation in format of videos can be found at: https://www.youtube.com/results?search_query=PBPK+model+developemnt, https://www.youtube.com/watch?v=Q3FAblNdnbU
Application of PBK models in risk assessment	Tan et al 2018, In addition available web-based presentation in format of videos can be found at: https://www.youtube.com/watch?list=PL7K5dNgKnawaBG_a9JHtLXDh1tr7RMK8O&v=5Lh4pJdEZcE
Selected examples of chemical assessments where PBK models were used	Perchorate, US EPA 2014 https://pubmed.ncbi.nlm.nih.gov/23901895/ https://pubmed.ncbi.nlm.nih.gov/17454566/ Acrylamide, EFSA 2015 https://efsa.onlinelibrary.wiley.com/doi/pdf/10.2903/j.efsa.2015.4104 Bisphenol A, EFSA 2015 https://efsa.onlinelibrary.wiley.com/doi/pdf/10.2903/j.efsa.2015.3978 Estragole, EMA 2020 https://www.ema.europa.eu/en/documents/other/second-draft-revision-1-public-statement-use-herbal-medicinal-products-containing-estragole_en.pdf

1.4. Comparison with other guidance documents on PBK models

This guidance builds on the accepted best practices of PBK model development and application that are reported in other documents. The most relevant documents are summarised in the following paragraphs.

In 2006, the US Environmental Protection Agency (EPA) published a document entitled "Approaches for the Application of Physiologically Based Pharmacokinetic (PBPK) Models and Supporting Data in Risk Assessment". This document provides an overview of the rationale for using PBK models in risk assessment, the main model characteristics to review for risk assessment purpose, and applications of PBK model simulations within the EPA risk assessment framework (EPA, 2006).

In 2010, the World Health Organization (WHO) International Programme of Chemical Safety (IPCS) published a guidance document on "Principles of Characterising and Applying PBK Models in Risk Assessment" to promote best practices and transparency in PBK modelling, and to facilitate understanding and sharing of these models in risk assessment reports (WHO, 2010). In addition, Meek et al. (2013) reported several case studies illustrating the approaches established in the WHO guidance document.

In 2014, the European Food Safety Authority (EFSA) published a scientific opinion on good modelling practice in the context of mechanistic effect models for risk assessment of plant protection products. The opinion identified several critical steps for using environmental models in risk assessment, such as problem formulation, model domain of applicability, selection of environmental scenario for pesticides, toxicokinetic characteristics, and species selection (EFSA, 2014).

In the pharmaceutical field, there have been ongoing efforts by the European Medicines Agency (EMA) and the US Food and Drug Administration (FDA) to standardise the

content and format of PBK submission. In 2018, the EMA, published a "Guideline on the Reporting of Physiologically Based Pharmacokinetic (PBPK) Modelling and Simulation" to provide detailed advice on what to include in a PBK modelling report and which supportive data are expected to qualify a PBK platform (EMA, 2018). The FDA also published a document titled "Physiologically Based Pharmacokinetic Analyses - Format and Content, Guidance for Industry" to outline the recommended format and content of a PBK modelling report submitted to the FDA (FDA, 2018). In addition, in 2020 new guidelines on the use of PBPK model´s simulations for drug development were published by the Japanese Pharmaceuticals and Medical Devices Agency (PMDA)/Ministry of Health, Labour and Welfare (MHLW).

1.5. Overview of the document

Chapter 2 (PBK modelling workflow) summarises the steps taken to characterise and validate PBK models using physicochemical and biochemical data from *in vitro* and *in silico* methods. Chapter 3 (purpose-specific assessment of PBK models) presents a template for reporting models and a checklist for evaluating their validity and applicability, according to context of use.

Appendices provide supplementary materials: Annex 1, list of resources for PBK modelling; Annex 2, prospective use of microphysiological systems in PBK models; Annex 3, sensitivity analysis details; and Annex 4, a list of the available case studies accompanying this guidance document, used as illustrative examples.

Notes

[1] Throughout this document we use the general term PBK model, treating this as synonymous with PBPK, PBBK and PBTK. See glossary for further explanation.

[2] Data-poor in the sense that *in vivo* kinetic data are lacking.

Chapter 2. PBK modelling workflow

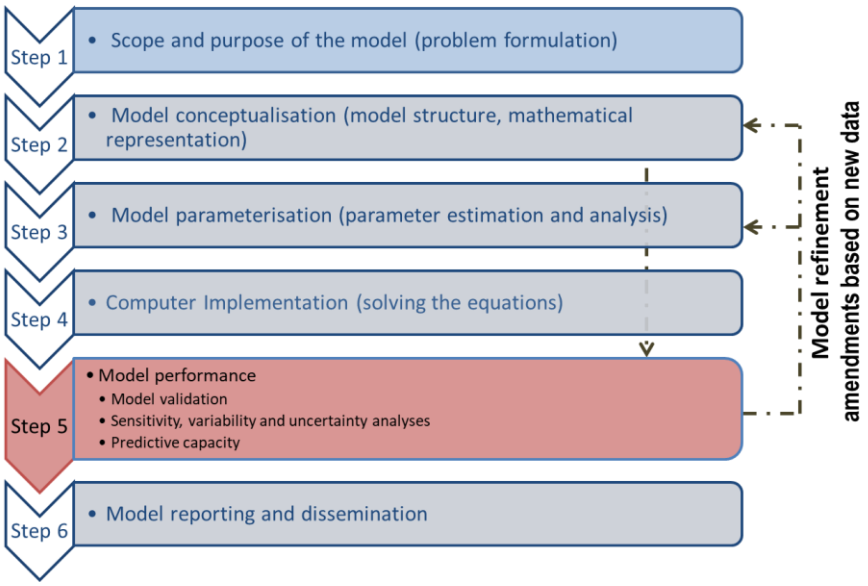
2.1. Introduction

Traditionally, during the development of a PBK model, some parameter values are determined by fitting model predictions to kinetic data from *in vivo* studies (e.g., plasma or target tissue concentrations over time). In such cases of model calibration, confidence in the predictive capacity of a PBK model is based partly on the concordance between model predictions and *in vivo* kinetic data not used to parameterise the model (e.g. WHO, 2010, EPA, 2006).

Given the trend to reduce animal testing in chemical risk assessment, PBK models have become an important tool to facilitate the translation of doses that elicit biological responses in cellular systems (often *in vitro*) to exposure levels *in vivo*. The challenge for the PBK modelling community is to parameterise models partially or entirely based on data from *in vitro* and *in silico* studies, with limited or no availability of *in vivo* kinetic data to parametrise/calibrate and to compare predictions.

This chapter provides an overview of a six-step modelling workflow (Figure 2.1) with emphasis on tools/methods available to parameterise PBK models in the absence of sufficient *in vivo* data for calibrating the model or assessing its predictive capacity. The workflow also allows refining the model based on amendments from new information (new *in vitro* data, new biological knowledge, etc).

Figure 2.1. Steps for PBK model development, validation, reporting and dissemination. In step 5 the performance of the model may be considered satisfactory for the intended use. However, if the model needs to be refined, this can be done based on new data or new knowledge, which will be an input to step 2 or 3.



2.2. Step 1 – Scope and purpose of the model (problem formulation)

Problem formulation is "a systematic planning of steps to identify the major factors to be considered in a particular assessment in relation to preliminary hypotheses with regards to hazard assessment (i.e. likelihood and severity of adverse effects which might occur or have occurred) and exposure assessment (i.e. likelihood and significance of exposure)" (EPA, 2006).

Problem formulation is an iterative process involving risk assessors and risk managers who determine the need for, and the extent of, a risk assessment (WHO, 2010). The expected time frame and resources also need to be considered (EFSA, 2017). It is important to ensure that the question(s) are clear, specific and agreed (EFSA, 2015). The question(s) need to fully encompass the issue(s) that need to be addressed, since the intended use of a PBK model, and the decision-making context, determine the required level of confidence in the model (EFSA, 2015). In this context, "intended use" refers to the scientific purpose of the model (e.g., generation of a dose-metric and its use in a risk assessment), while the "decision-making context" (or context of use) refers to relevant considerations (e.g. acceptable uncertainty, risk management consequences of making a decision, availability of existing data, possibilities to generate new data, restrictions/bans on animal testing). Examples of possible regulatory applications of PBK models are presented in Table 2.1.

Table 2.1. Possible applications of PBK models in chemical risk assessment.

1	Extrapolating across doses or exposure scenarios
2	Route to route extrapolation (of an external dose)
3	Interspecies extrapolation (and modification of default assessment factors)
4	Intraspecies extrapolation (accounting for population variability)
5	*In vivo* extrapolation of *in vitro* toxicity data – (Q)IVIVE
6	Setting safe levels of a chemical based on tissue dosimetry (in humans or animals)
7	Interpreting human and wildlife biomonitoring data by retrospectively reconstructing the external dose or exposure (reverse dosimetry)
8	Predicting biologically-relevant doses at target tissues
9	Bioaccumulation assessment

2.3. Step 2 – Model conceptualisation (model structure, mathematical representation)

The structure of a PBK model (the conceptual model) is informed by the problem formulation, knowledge of the underlying biokinetic mechanisms, and the availability of suitable data. The level of detail in model structure relates not only to the selection of compartments (e.g. whole body, target tissue or intracellular concentrations), but also to

the chemical forms that are tracked within the model (i.e. parent chemical and/or metabolite(s)). Evaluation of existing literature and data are the inputs to model conceptualisation.

When selecting a PBK model for a regulatory application, the principle of model parsimony should be followed, i.e. the model should be only as complex as necessary to address the assessment in a fit-for-purpose manner. In some cases, a simple one-compartment model that describes the uptake and clearance of a chemical may be sufficient to estimate systemic concentration from exposure to a given dose. In other cases, a PBK model that consists of two or more discretely defined organ/tissue compartments is needed, for example, to include a target tissue when translating an *in vitro* result measured using cells/enzymes from the target tissue (e.g. hepatocytes, thyroid peroxidase). In addition to the intended use of the model, physicochemical properties of the chemical and available biochemical data (e.g., metabolic rate constants, protein binding/adduction) are key factors to inform a model structure, including the number and types of compartments, exposure routes, and ADME characteristics.

In general, chemical partitioning into compartments is typically assumed to be instantaneous and chemical composition throughout a given tissue, homogeneous ("well-mixed") (Thompson et al., 2011; Thompson & Beard, 2012). Organs and tissues that are assumed to be "kinetically homogeneous" are lumped into a compartment; in other words, any kinetic changes in these individual organs/tissues are so fast that the relative amounts of a chemical in these organs/tissues stay the same. It is also assumed (Krishnan & Andersen, 1994) that there is venous equilibration (i.e., the free tissue concentration and the venous concentration exiting the tissue are equal) and within each compartment there is a similar disposition (i.e., blood flow rate divided by the product of partition coefficient and tissue mass). When lumping or splitting compartments, the following principles should be observed: 1) the sum total of tissue and organ compartment masses should be within the body mass of the organism; and 2) the total blood flow (i.e. cardiac output) in the model should be equal to the sum of the flows to the tissue compartments of the model in order to maintain the mass balance of the chemical at all times.

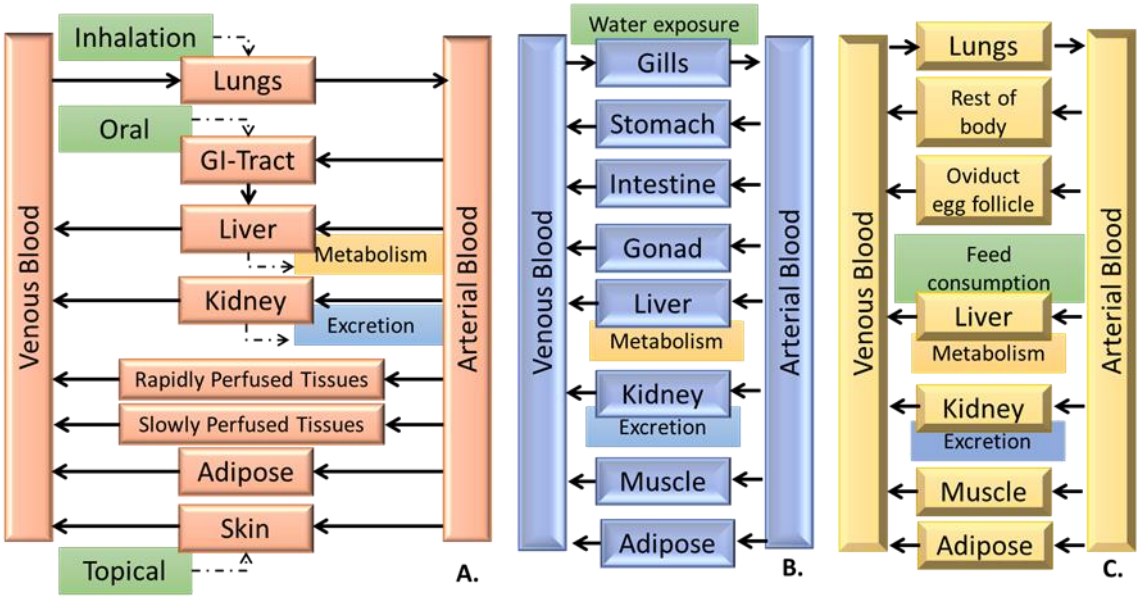
The chemical distribution to each compartment is either perfusion-limited or permeability-limited uptake (Jones & Rowland-Yeo, 2013). In perfusion-limited uptake, blood flow rates are the limiting process. In permeability limited uptake, permeability across the cell membrane is the limiting process. In this case, the tissue is divided into essentially two compartments, representing the intracellular space and the extracellular space, which are separated by a cell membrane that acts as a diffusional barrier. In permeability-limited uptake, permeability across the cell membrane is the limiting process; this tends to occur for larger, polar molecules. Active transport could be also involved and modelled by incorporating uptake parameters into a permeability rate-limited model (Jones & Rowland-Yeo, 2013).

Examples of schematic PBK models are given in Figure 2.2. Figure A represents human or mammals (rodent, horses, pig, cattle, wildlife); the model includes three exposure routes (inhalation, oral and dermal/topical), discrete compartments for liver, lungs, kidneys, adipose tissue, and two lumped compartments; a rapidly perfused group, comprising organs such as adrenals, brain and heart and a slowly perfused group, comprising organs and tissues such as muscles. More refined and complex models may include the foetus as a compartment, or include more detailed metabolic pathways, such as phase I and phase II metabolic processes. Figure B represents a PBK model for fish, with the gills highlighted as the main organ in contact with contaminated water, and other relevant compartments

such as gonad, liver (for metabolism), kidney (for excretion) and adipose tissue (for storage). Similarly in Figure C, representation is for poultry (birds) with the main exposure route being feed consumption linked directly to liver with the addition of oviduct for egg deposition.

PBK models for fish and other species, such as poultry, have also been developed and can be parameterised with *in silico* and *in vitro* data from the specific species (see case studies 1 & 2). In an environmental chemical assessment, these more complex models may prove useful for higher tier bioaccumulation assessments.

Figure 2.2. Schematic representations of PBK models. A. Human/Mammals, B. Fish, C. Poultry.



2.4. Step 3 – Model parameterisation (parameter estimation and analysis)

A PBK model contains two types of parameters:

1. anatomical and physiological parameters for the species of interest (e.g. human, horse, cat, dog, cow, fish, rodents, poultry, etc.), such as tissue volumes and blood flow rates;
2. chemical-specific parameters, such as partition coefficients, rates of absorption (K_a), biotransformation - metabolic rate constants (e.g. Intrinsic Clearance [Cl_{int}], maximal rate [V_{max}] and concentration at half-maximal rate [K_m]), macromolecular binding (e.g. unbound fraction [fu] and DNA adduct formation) and excretion of compounds (e.g. elimination rate [K_{elim}]). These parameters may be measured using *in vitro* methods, or estimated based on physicochemical properties of the chemical, such as molecular weight, octanol-water partition (K_{ow}), octanol-air partition (K_{oa}). In certain cases, scaling factors may be applied to extrapolate and scale up the *in vitro* experiment parameter to organ/body unit.

Physiological and anatomical parameters can be found in the literature (Brown et al., 1997, Davies and Morris 1993), and in online databases, such as the Dutch Interspecies Database (https://www.interspeciesinfo.com). If a parameter is not available for the species of

interest, as reviewed in Madden et al (2019), it can often be estimated from another species by using allometric scaling. This is an empirical approach based on the assumption that the underlying physiological processes (such as cardiac output, heartbeat frequency, breath duration) are related to the body size (West et al., 1997). Care must be taken when applying these algebraic scaling laws in cases where they are entirely weight-based scaling factors, and do not account for species differences in the underlying processes, such as metabolism and active transport (Hall et al., 2012). Possible additional inaccuracies that can be associated with the use of allometric scaling are discussed in Rowland et al (2011). Species-specific anatomical and physiological *in vivo* parameters are valuable pieces of information to support development and use of PBK models.

Chemical-specific ADME parameters can be obtained from a range of resources, including both databases and models, such as those listed in Annex 1. An overview of *in silico* and *in vitro* methods for generating human ADME parameters is provided by Bessems et al. (2014). In case ADME parameters are estimated using *in silico* methods, several guidance documents are available for evaluating the quality of some *in silico* methods. For example, the OECD has defined five principles for validating Quantitative Structure-Activity Relationship (QSAR) models, which apply to QSARs based on structural features and/or physicochemical properties (OECD, 2007): 1) a defined endpoint; 2) an unambiguous algorithm; 3) a defined domain of applicability; 4) appropriate measures of goodness-of–fit, robustness and predictivity; and 5) a mechanistic interpretation, if possible. At present, however, relatively few QSAR models for ADME parameters have been documented according to the OECD validation principles. This does not necessarily mean that these models are not valid, but there may be less confidence in applying them, due to lack of documentation relating to one or more aspects of the accepted principles.

In case parameters are measured, several documents are available to provide guidelines (OECD TG 428; OECD TG 319 a, b) on generating reproducible and reliable *in vitro* data. In the absence of standardised *in vitro* methods, *in vitro* ADME data should be generated in accordance with the OECD guidance document on "Good *in vitro* Method Practices" (GIVIMP) (OECD, 2018).

It is very important to report in a transparent way how the *in silico* and *in vitro* data were calculated and measured to ensure that quality in model input is high. In general, experimentally measured values should be given preference over predicted parameters. Also, it should be clarified how non-detects were treated (zero, 50% of LOD, LOD, range, no data).

Challenges in describing the *in vitro* kinetics in the culture medium, where the stability of the test chemicals would affect the cellular responses, and mimicking the actual chemical kinetics in cells in the target tissues under real-world exposure scenarios should be taken into account. To extrapolate between the nominal concentration applied in *in vitro* experiments to the free concentration and the effective intracellular concentration, various *in vitro* fate and dosimetry models have been developed, such as the Armitage model, the Virtual Cell Based Assay (VCBA), and the Virtual *in vitro* distribution (VIVD) model (Kramer, 2010; Armitage et al. 2014; Zaldivar et al., 2017; Fischer et al. 2017; Fisher et al., 2019). To date, this type of modelling approach has supported the design and interpretation of *in vitro* experiments but has not been used in regulatory assessments. This approach is regarded as an extension of traditional PBK modelling to cellular and subcellular compartments, and thus in principle covered in the scope of this guidance.

Current *in vitro* methods are limited in recapitulating physiological characteristics, and this is especially relevant for the most refined PBK modelling. It is expected that advances in

the development of micro-physiological systems (tissue and organ-on-chip devices) will benefit the development of more complex PBK models (see Annex 2 for further details). However, this lies outside the scope of the current guidance, since the technology is rapidly evolving, and experience still needs to be gained on how to characterise and validate such systems and how to link them to PBK models.

The subsections below highlight some important considerations for the model developer when determining values for the most common chemical-specific parameters using *in vitro* or *in silico* "non-animal" methods. Key issues are also identified as pointers for the assessor.

2.4.1 Absorption across external barriers

Absorption is the process by which a chemical enters the body, the major routes being oral, dermal and inhalation. For therapeutic drugs intravenous injection and oral exposure are most common. The absorption process depends on both passive diffusion across the epithelial barrier as well as active uptake and efflux.

For chemicals or drug exposure for fish species absorption occurs through the gills assuming steady state exposure to a chemical via water (e.g., factoring in flow rate of water through the gills and the blood-water partition coefficient).

In the case of the oral and dermal exposure, the chemical mainly enters the venous blood, However, in the case of inhalation; exposure will be first via arterial blood since lungs are oxygenating the body. Case study 12 describes the application of PBK modelling in the next generation risk assessment of dermally applied consumer products.

OECD TG 428, test method has been designed to provide information on absorption of a test substance, (ideally radiolabelled), applied to the surface of a skin sample separating the two chambers (a donor chamber and a receptor chamber) of a diffusion cell.

In the risk assessment approach, mainly the extent rate of absorption is used (i.e. percentage oral/dermal/inhalation absorption) instead of a flux rate as reported in OECD Guidance Notes on dermal absorption No 156 (ENV/JM/MONO(2011)36) and EFSA Guidance on dermal absorption (EFSA Journal 2017;15(6):4873). Usually data submitted for risk assessment are expressed as proportions (e.g. % values), while flux rate should be considered to enable the implementation of a PBK model in regulatory risk assessment; however, this document will not give guidance on how to appropriately convert rate/flux and extent/percentage, this should be part a new proposal/effort.

Background information on absorption parameters is given in Box 2.1 and Table 2.2, while guidance is given in Table 2.3.

Box 2.1 Background on absorption

Expression of the parameter

Absorption is typically expressed as a rate (flux across a barrier), passive concentration–dependent crossing of external epithelia (Bessems et al., 2014).

For oral absorption, the key parameter is permeability (distance travelled over time). This is obtained by taking the transport rate divided by the surface area of the membrane or assay. When permeability is measured using *in vitro* assays, it is referred to as the apparent permeability coefficient (P_{app}); usually the unit is cm·h^{-1} (mostly *in vitro* data is cm·10^{-6}/s). Likewise, when permeability is measured *in vivo* in animals or humans, it is referred to as effective permeability ($P_{eff,}$ cm·h^{-1}).

For dermal absorption, typically the apparent dermal permeability or penetration coefficient P_{app} ($K_{p,app}$)c, cm·h^{-1} is used. This parameter can be complemented with maximum flux (j_{max}, µg.cm^{-2}.h^{-1}), dermopharmacokinetic parameters (partition coefficient stratum corneum/vehicle and diffusion coefficient in stratum corneum) as well as others parameters describing the behaviour of the chemical into the skin.

For inhalation, the apparent inhalation permeability or penetration coefficient is P_{app} (example, cm·h^{-1}).

Table 2.2 Methods to measure or predict absorption

Absorption route	Techniques	Comments	Reference
External absorption			
Oral* Most common modelled route of exposure	Computational models of absorption rely on a variety of *in vitro* and/or *in silico* input data, such as solubility, permeability, particle size, logP (logKow), and pKa to simulate the kinetics associated with dissolution, precipitation, uptake, and absorption of a compound as it transits through the gastrointestinal tract.	Oral uptake is generally described by an absorption rate constant calculated from the effective permeability (i.e. permeability scaled from the *in vitro* to *in vivo* situation)	Hansch et al., 2004; Zhao et al., 2003; Kamiva et al., 2019.
Topical (dermal)**	Computational techniques range from linear models based on simple physicochemical parameters to more complex models representing the diffusion and partitioning of compounds through the different phases of skin tissue. Experimentally, the use of ex vivo skin to measure the absorption rate of topically applied chemicals in static or flow-through cells is widely accepted with OECD guidance available for these methods (OECD TG 428).	As the skin absorption study protocol match as much as possible to the real conditions of use (formula, amount of formulation, exposure time, anatomical area), there was less than 2-fold difference between the *in vitro* and *in vivo* results. *In silico* tools for prediction of dermal absorption of pesticides and to highlight that further work is needed to better understand the effect of co-formulants	Potts and Guy 1992; Mitragotri et al., 2011; Shen et al 2014. Wang et al. 2006, Chen et al 2015, Kneuer et al. 2018. Lehman et al. 2011. Kneur et al., 2018
Inhalation	Apparent inhalation permeability and deposition rates in the airways can be predicted using several computational tools. *In vitro*	Inhalation absorption is mainly applied to study volatile chemicals, aerosols, and particulates.	reviewed in Rostami 2009. more details in

	models available for inhalation are: headspace models, Solid-phase micro extraction (Kba), submerged cell lines, Air–liquid interface assays and 3D models		Bessems et al., 2014).
Internal absorption			
small and large intestines***	Advanced dissolution, absorption and metabolism (ADAM) or advanced compartmental absorption and transit (ACAT) model	Mechanistic models sub-sections, each parameterized with specific physiological data such as lumen diameter, surface area and transporter/metabolic enzyme expression.	Huang et al., 2009
Fish absorption			
Gills	*In vitro*, *in silico* techniques are available to obtain this parameter	The gill is the principle site of xenobiotic transfer to and from the aqueous environment	Nichols et al, 2007 Stott et al 2015; Chang et al., 2018

*Oral uptake in each segment will be predicted with an absorption rate coefficient specific to that segment using the effective permeability parameter (i.e. *in vitro* to *in vivo* extrapolation; IVIVE). Depending on the level of detail, mechanistic models can be included to account for metabolism in the gut, transporter-mediated uptake or efflux of chemicals and the effect of bile dissolution of chemical. This approach is in contrast to empirical models when *in vivo* kinetic data are available to calibrate a single absorption rate constant to the entire length of the intestine and does not explicitly account for metabolism or transport in the intestines.

** Is important to consider that for absorption, especially for dermal absorption, the formulation ingredients and the physical-chemical properties of the formulation / vehicle / matrix impact the extent of absorption, and should be reported. Only in few cases or for industrial uses humans/animals are exposed to pure substance, generally exposure is to mixtures.

***Effective permeability can be measured *in vivo* using the Loc-I-Gut method (Lennernäs et al., 1992), or more commonly, predicted from *in vitro* data. This is accomplished by scaling *in vitro* apparent permeability measured using cell-based systems (MDCK, Caco-2) or cell-free systems (PAMPA) to *in vivo* effective permeability using linear regression relationships derived between the *in vitro* system and *in vivo* Loc-I-Gut data obtained for a common set of compounds (Winiwarter et al., 1998; Sun et al., 2002).

Table 2.3. Absorption parameters – pointers for the modeller and assessor

Issue	*What to do**
Mass transfer limitations can be different between the *in vitro* and *in vivo* situations (permeability vs perfusion limited).	If possible report the difference in fold or apply correction factor.
For dermal route : The effect of the formulation /vehicle can affect the absorption rate or K and D parameter	The formulation and vehicle used for testing can affect experimental output values. Parameter influenced by the formulation/vehicle must be evaluated in the same condition (same formula, applied dose, time of application…)
A high variability of parameter values results from various *in vitro* systems e.g. derived apparent permeability coefficients (Papp) values due to the experimental conditions of the test applied (Caco-2, PAMPA, OECD TG428). *In vitro* systems such as Caco-2 have significant inter-lab variability (Lee et al., 2017).	It is highly recommended that Caco-2 data should be first calibrated using a common set of reference compounds, including those with low, mid and high permeability values.
Knowing if the chemical goes into enterohepatic recirculation.	Residual uncertainty*
Cell-based intestinal uptake measurements affected by the degree of ionization for acids and bases.	Cell-based intestinal uptake measurements should be performed using pH values specific to that segment (e.g. pH 6.5 for duodenum), as this affects the degree of ionization for acids and bases.
Types of cells used in *in vitro* studies can also affect the absorption parameters	An *in vitro* approach requires the right type of cells for assays i.e. those that can closely mimic the physiological environments *in vivo*. Scott and Ramsey (1987) found that cypermethrin did not penetrate *in vitro* through whole skin but did penetrate epidermal membranes (Comparison of the *in vivo and in vitro* Percutaneous Absorption of a Lipophilic Molecule (Cypermethrin, a Pyrethroid Insecticide, Scott and Ramsey 1987).
*In cases where there is a residual uncertainty, which cannot reasonably be reduced through additional data generation, this uncertainty should be recorded. Wherever possible, an attempt should be made to qualitatively express the impact of this uncertainty, for example whether the assessment is expected to be more or less conservative as a result.	

1.1.2. Partitioning

Partition coefficients are essential determinants to describe the distribution of chemicals within an organism. Partition coefficients define the gradients that drive the passive exchange of a chemical between the organism and its environment, as well as the passive exchange between blood cells and plasma, and between the plasma and all compartments (tissues/organs) in the organism.

The sorption capacity of biological tissues is related to their composition in terms of a limited number of components, namely storage lipids (triglycerides), phospholipids,

lysosomes, various proteins and water. Several *in vitro* and *in silico* methods are available to address tissue-specific partition coefficients (Table 2.4).

For many chemicals, uptake into and efflux out of tissues is dependent on the action of membrane transporters as well as passive partitioning (see 2.4.3).

Background information on partition parameters is given in Box 2.2 and Table 2.4, while guidance is given in Table 2.5.

Box 2.2. Background on partition coefficients

Expression of the parameter
A tissue-plasma partition coefficient is defined as the ratio of the tissue concentration to the arterial plasma concentration at equilibrium.

Table 2.4. Methods to measure or predict the Partition Coefficient parameter

Partition Coefficients (PC)	Techniques	Comments	Reference
*In vitro** tissue-plasma coefficients (include measures of lipophilicity, plasma protein binding, pKa and blood-plasma partition ratio)	Several *in vitro* techniques are available for measuring protein binding including ultrafiltration, ultracentrifugation, equilibrium dialysis and surface plasmon resonance, and thermophoresis.	There are benefits and drawbacks to each of the methods, although equilibrium dialysis is the most widely used technique in the pharmaceutical industry.	Smith & Waters 2018; Smith et al 2010; Bohnert & Gan 2013.
*In silico*** Tissue-specific coefficients (such as: blood:air (Pba), tissue:air (Pta), tissue:blood (Ptb), tissue:plasma (Ptp), skin:water (Pskw))	(Q)SAR models and biologically-based algorithms or a combination	Is based on chemical structure or properties. *In silico* tissue-plasma partition coefficients have been predicted based on the logKow*** partition coefficients of the respective chemicals using calibrated empirical relationships	Poulin and Krishnan, 1996 a,b; Poulin and Theil, 2000; Buist et al., 2012; Rodgers and Rowland, 2006; Endo and Schmidt, 2006; Schmitt, 2008; Peyret et al., 2010; Adler et al. 2011; Sarigiannis et al. 2017.
In silico membrane-water coefficients. (For neutral and ionic chemicals)	Based on their molecular structure, has been put forward based on the COSMO-RS theory	Recommended to experimentally measure these parameters to reduce the compounded degree of uncertainty.	Bitterman et al, 2014, 2016

* An overview of existing experimental data, methods and prediction tools for neutral chemicals can be found in (Howard and Muir, 2010; Hodges et al., 2019; Vitale and DiGuardo 2019).

***In silico* predictions of lipophilicity, pKa and protein binding can also be used in place of *in vitro* measurements (Emoto et al 2009; Toma et al 2019). While these methods work well for neutral organic molecules, research with organic ions suggests that logKow based prediction methods are less reliable (Hodges et al., 2019; Vitale and DiGuardo 2019).

***For neutral chemicals, a more mechanistic approach has been established on the basis of interaction energies (Endo et al 2013; Endo & Goss, 2014; Henneberger et al 2016a,b; Endo et al 2011, Rodgers et al., 2005; Rodgers & Rowland, 2006).

Table 2.5. Partitioning parameters – pointers for the modeller and assessor

Issue	*What to do**
Attention should be paid to the reliability of methods used to determine partitioning ($logK_{ow}$) for organic ions. For instance, if incorporating predictions for $logK_{ow}$, the values will be dependent on method/software/version used which must be explicitly recorded in addition to overall partitioning prediction.	1. OECD TG 107 (Shake flask method) and TG 117 (HPLC method) are available to measure the LogKow partition coefficient. 2. When available, use measured values. 3. Check the validity of the QSAR model used for predicting the value.
The applicability domain of models needs to be considered. For example, the model by Rodgers and Rowland refers to pharmaceutical compounds within a certain range of $LogK_{ow}$, pKa and plasma unbound fraction values.	Highlight tissue-plasma partition coefficients that are predicted for chemicals that are outside of the applicability domain of the model used to generate them.
In the case of chemicals that are highly bound to proteins, it can be difficult to reliably determine the unbound fraction and distinguish the unbound fraction from zero.	Highlight chemicals that are highly bound to proteins. As reported by EMA, FDA and PMDA a minimum value for fraction unbound (fu) should be 0.01** (EMA, 2013; FDA, 2020; PMDA, 2018).
It should be considered whether active transport can be excluded between systemic circulation and organs/tissues/compartments.	Permeability-limited models coupled with transporter kinetics for specific organs (like hepatic and renal, mentioned in 2.4.3) should be incorporated into the PBK model when necessary in order to accurately predict the plasma concentrations of a chemical.
*In cases where there is a residual uncertainty, which cannot reasonably be reduced through additional data generation, this uncertainty should be recorded. Wherever possible, an attempt should be made to qualitatively express the impact of this uncertainty, for example whether the assessment is expected to be more or less conservative as a result. ** this is for conservative Drug to Drug Interaction (DDI) prediction as perpetrator. Although, this is the option available, it might be inappropriate to apply fu of 0.01 leading to overestimation when dealing with environmental chemicals.	

2.4.1. Active transport

Transporters can influence the absorption, distribution and excretion of a chemical. Active transport is especially important for chemicals with low passive permeability, thereby mediating the penetration of these chemicals into and out of tissues (see case study 7).

In the context of absorption, transporters are found in barrier tissues such as the intestinal enterocyte (Kunta et al., 2004; Boudry et al., 2010) and pulmonary epithelial cells (Bosquillon 2010), where they facilitate the uptake of chemicals from the apical membrane of the enterocyte or epithelial cells. Efflux transporters found on the basolateral membrane of the cells work in concert to transport the chemical out of the cells and into the blood, thereby facilitating absorption. In contrast, efflux transporters found on the apical membrane transport chemicals back into the intestinal lumen or epithelial lining fluid, reducing the absorption of the chemical.

Once present in the systemic circulation, for many chemicals, uptake into and efflux out of tissues is dependent on the action of membrane transporters as well as passive partitioning (Kim et al, 2017; Jones et al, 2012; Jamei et al, 2014). Transporters are found on numerous

organs such as the brain, liver and kidney, where they influence the penetration of chemicals into the organ (distribution) (Ayrton & Morgan 2001). This in turn can influence other processes such as metabolism. For example, uptake transporters deliver chemicals with poor passive permeability into the hepatocytes for subsequent metabolism. Conversely, sinusoidal efflux transports limit the contact of chemicals with moderate passive permeability with liver enzymes.

Differences in expression of transporters between fresh tissue versus isolated/cultured cells versus recombinant systems should be considered (Vildhede et al., 2015), as down-regulation of transporters can occur when primary cells are in culture, while recombinant systems can lead to overexpression of transporters relative to the true *in vivo* expression in tissues. Absolute quantification of transporter expression in *in vitro* systems used for measurement should be performed in-house whenever possible with proteomics-based approaches following the latest guidelines (Qiu et al., 2014; Prasad et al., 2019). Published data in the literature concerning absolute expression levels of transporters in various tissues may be used to support the derivation of the scaling factors (Vildhede et al., 2015; Harwood et al., 2019; Prasad et al., 2016). It has been demonstrated that robust IVIVE can be achieved using expression-based scaling factors for transporter-dependent chemicals (Chan et al., 2019).

Finally, transporters are crucial for the elimination of some chemicals from the body via the liver (hepatobiliary excretion) or kidney (renal excretion). This process facilitates the removal of both metabolites and unchanged chemicals from the body (Shitara et al., 2006). The opposite can occur, where transporters could also lead to an accumulation of chemicals in the body. Uptake transporters found in the renal tubules of the kidney permits the reabsorption of certain chemicals back into the tubular cells and blood stream, prolonging the half-life of their substrates.

Transporters can play an important role in the *in vivo* kinetics of chemicals but the science is not currently at a state where this can be routinely or reliably incorporated into PBK models. This is a challenge for pharmaceutical compounds that have a lot of existing biokinetics data and even harder for non-pharmaceutical compounds that are data poor. Pure *in vitro* based evaluation of relevance of transporters is difficult and still not routinely established. Experimental work is extremely time and capacity intensive and the evaluation of results difficult without the possibility to compare to *in vivo* data. Nevertheless, table 4 provides some guidance to address the question of transporters. Such recommendations have to be revised according to evolution of the state of science.

Background information on active transport parameters is given in Box 2.3 and Table 2.6, while guidance is given in Table 2.7.

Box 2.3 Background on active transport

Expression of the parameter

Transporter activity may be expressed as an intrinsic clearance term (mL/min). This is obtained from the quotient of the rate of transport and the concentration of the chemical. Alternatively, the saturable kinetics of a transporter can be expressed as J_{max} and K_m, the maximal transport rate (mol/min) and affinity constant (mol/L) respectively. K_m is a constant for the same transporter and substrate pair regardless of the *in vitro* system used for measurement. Intrinsic clearance, refers to unbound compound, and can also be derived by dividing J_{max} by K_m.

Table 2.6 Methods to measure or predict active transporters

Transporter kinetic parameter Method*	Techniques	Comments	Reference
In silico	Various permeability limited models for the liver, kidney, intestine and brain have been published	These models can be parameterised with the kinetic parameters obtained from different *in vitro* systems.	Jamei et al., 2014; Huang & Isoherranen 2018; Neuhoff S. et al. (2013) Huang et al., 2009; Gaohua L et al., 2016
In vitro systems** - several *in vitro* systems have been used to study the transport of chemicals.	• isolated cells, (e.g. hepatocytes (Mao et al, 2018) and proximal tubules (Worley and Fisher, 2015), • sandwich cultured hepatocytes (Jones et al, 2012), • cell lines (Kumar et al, 2018) and • recombinant expression systems.	Depending on whether the *in vitro* system expresses multiple transporters (hepatocytes, proximal tubules, Caco-2) or a single transporter (single-transfected cells, membrane vesicles), the kinetic data obtained would be interpreted differently. Whenever possible, the fraction unbound of the chemical in the transport medium or intracellularly should be measured as only the unbound drug interacts with the transporter binding site (Obach et al., 1996) ***	Trapa et al, 2019; Guo et al, 2018; Yao et al, 2018. A summary of the different *in vitro* systems available and their applications and limitations are found here (Brouwer et al., 2013).
In vitro to in vivo extrapolation – scale up to organ	This can be achieved by using various scaling factors that account for differences in transporter expression or activity between the *in vitro* system and *in vivo* tissues	When converting *in vitro* transporter kinetics to *in vivo* transporter kinetics within the PBK models, it is important to account for the mechanistic differences between the *in vitro* system and *in vivo* organs.	(Chan et al., 2019)
In vitro and ***in silico*** models for chemical-transporter interactions chemical-chemical. Interactions or chemical-drug interactions to occur at the transporter binding site.	Several methods reported in the review	When a chemical is a substrate for several notable transporters such as the OATPs, OCTs, P-gp and BCRP, chemical-drug interaction assays should be conducted with a known inhibitor following the latest FDA guidelines (FDA, 2020).	Clerbaux et al (2019).

*To mechanistically account for the action of transporters in PBK models, it is necessary to use a permeability limited organ structure and to account for both passive permeability and active transport the chemical of interest.

**The experimental conditions of the *in vitro* system must be carefully defined. It has been observed that the pH (Varma et al., 2011) and albumin concentration (Kim et al., 2019) of the medium would impact the kinetic parameters obtained, particularly with organic acids.

***Intracellular concentrations can either be measured experimentally, or analysed using an *in silico* compartmental model. The latter should be used particularly when analysing the data obtained from efflux transporter assays (Zamek-Gliszczynski 2013).

Table 2.7. Active transport parameters – pointers for the modeller and assessor

Issue	***What to do****
Transport parameters generated using *in vitro* systems often differ to the *in vivo* situation (e.g. viability of *in vitro* system may decline with time, differences in transporter density and number)	Scaling factors could apply.
For some tissues, there is limited knowledge of the transport processes and limited understanding of their toxicological relevance.	Residual uncertainty*
A historical inconsistency of the nomenclature for transporters	Residual uncertainty*
Transporter measurements using intrinsic clearance cannot account for saturable kinetics.	When possible, V_{max} and K_m terms should be measured.
Most PBK models assume that simple Michaelis-Menten kinetics will sufficiently describe the kinetics of the transporter. But this may not necessarily be true for all transporters, for example, SLCO2B1/OATP2B1 is known to have two substrate binding sites whose kinetic parameters vary with pH as well. This is important particularly in the intestines where the pH values can vary from 6.5-8.5, resulting in different innate transporter activities in different segments of the intestine.	Measurements should be made using physiologically-relevant pH values. Mathematical models that are not based on Michaelis-Menten kinetics can be applied.
Transporter location and polarity	The location of the transporter and polarity (i.e. apical or basolateral, uptake versus efflux) should be clearly defined. Measurements should be made using physiologically-relevant pH values.
Intrinsic activity of transporters	It is assumed that the (normalised) intrinsic activity of transporters remains the same between the *in vitro* and *in vivo* system when using scaling factors that account for differences in expression levels of transporters. Furthermore, it is assumed that all the transporters quantified are functionally active, but they probably have different rate, particularly in transfected/recombinant systems.
*In cases where there is a residual uncertainty, which cannot reasonably be reduced through additional data generation, this uncertainty should be recorded. Wherever possible, an attempt should be made to qualitatively express the impact of this uncertainty, for example whether the assessment is expected to be more or less conservative as a result.	

2.4.2. Systemic Clearance

Systemic Clearance refers to the removal of a chemical from the systemic circulation. There are several mechanisms by which a chemical may be cleared although not all of them will make significant contribution. Sufficiently volatile chemicals may be cleared predominantly through exhalation; hydrophilic chemicals with low permeability are typically excreted as the parent chemical via the kidneys; and non-volatile hydrophobic chemicals are typically excreted as more water-soluble products of metabolism. The clearance rate depends on excretion and/or metabolism. Sometimes hepatic clearance reflects the overall clearance (see case study 3).

It is not always necessary to parameterise every clearance mechanism for a given chemical. For risk assessment purposes, it is conservative to assume zero contribution for minor clearance mechanisms, since this results in more of the parent chemical (if assumed to be the toxic moiety) being bioavailable in the plasma. Sometimes the minor clearance mechanism may be the one that is driving the association in epidemiology studies (Ruark et al., 2017).

It is of importance to include a justification of whether kinetic processes are included in the PBK model using parameters that describe saturable processes (e.g. using K_m and V_{max} for metabolism) or using parameters that assume linearity over the dose-range of interest (e.g. using Clint for metabolism). This is to avoid not accounting for potential saturation of certain kinetic processes when extrapolating over different doses. It should therefore be verified beforehand what the dose-range of interest is from the viewpoint of the regulatory needs that are to be addressed. For instance, for the identification of the intrinsic hazard of chemical or when addressing an accidental exposure, high exposures may need to be addressed by the model, including potential saturation kinetics involved.

Fish *in vitro* methods to measure clearance have the potential to provide important data for bioaccumulation Assessments, application of these systems can help in prediction of *in vivo* rates of metabolic clearance. The OECD TG 319 describes the use of cryopreserved rainbow trout (*Oncorhynchus mykiss*) hepatocytes (RT-HEP) as a metabolising system to determine the clearance (CL) of a test chemical using a substrate depletion approach. The value obtained can then be used to improve *in silico* predictions of the test chemical bioaccumulation in fish as shown in Nichols et al. (2013). These *in vitro* data can be used as strategy for *in vitro-in vivo* extrapolation of measured biotransformation rates and incorporation of estimated hepatic clearance into appropriate computational models (Nichols et al., 2006). The model may then be used to simulate the substance concentration any given species and predict a steady-state bioconcentration factor (BCF).

Background information on clearance parameters is given in Box 2.4 and Table 2.8, while guidance is given in Table 2.9.

Box 2.4 Background on clearance

Expression of the parameter

Usually, clearance is measured in units of flow (L/h or mL/min) and may be normalized by body weight. The quantity reflects the rate of elimination of the chemical divided by its plasma concentration.

Classification schemes

Several classification systems have been published to provide guidance on what is likely to be the predominant clearance mechanism for a particular chemical (Camenisch et al, 2016). These classification systems typically take as inputs physicochemical parameters (such as molecular weight, ionisation species and permeability) and return as output the predominant clearance mechanism or rate limiting step in clearance (expressed in categorical or probabilistic terms).

The Biopharmaceutical Drug Disposition Classification System (BDDCS; Benet, 2013) classifies chemicals into four classes according to their permeability and solubility. Compounds with high intestinal permeability (Class 1 and 2) are mainly eliminated by metabolism while others (Class 3 and 4) are often eliminated unchanged by biliary or renal excretion. Another scheme, the Extended Clearance Classification System (ECCS; Varma 2015), indicates whether clearance will be rate-limited by renal excretion of the parent, enzymatic metabolism or hepatic transport.

These schemes help to promote an understanding of the processes that should be included in a PBK model, and also guide the most appropriate experimental data to generate. For example, if a chemical's clearance depends primarily on metabolism but the rate limiting step is transport into hepatocytes, using isolated enzymes or a subcellular fraction to provide experimental data on clearance may result in misleading data and poorer predictions than using a whole cell-based model.

Table 2.8 Methods to measure or predict clearance parameter

Clearance	Description	Reference
Metabolic clearance*	Parameters describing the metabolic clearance of chemicals can be derived from a number of different *in vitro* systems. The most appropriate system is largely chosen based on the chemical under investigation, the metabolic pathways it undergoes, and the organ and sub-cellular location of the enzymes involved. The major site of metabolism for the majority of compounds is the liver, with the intestine playing an important metabolic role during first pass for some compounds. The blood/plasma can be a major site of metabolism for some chemicals such as esters, with the kidneys, lungs and other organs contributing to the overall clearance of some compounds. Skin metabolism also plays an important role for dermal exposure.	See case studies 5 & 6.
In silico	At present, structure-based computational methods are useful for simulating possible metabolic pathways, but the ability to quantitatively predict metabolic rate constants or enzyme affinity remain a challenge. If the production rate of a particular metabolite is of interest, there are predictive methods available for phase I reactions such as cytochrome P450 mediated reactions but the relevance of these methods is considered to be relatively poor compared to predictions of overall clearance. If such models are used, it is important to understand the level of confidence that is required (Chapter 3) and how much of a contribution they make to the overall PBK simulation. Where PBK model outputs are sensitive to these parameters and high confidence in results is required, *in vitro* methods should be considered to reduce uncertainty of the values for these parameters.	Pirovano, 2015; Zakharov et al., 2012; Stepensky et al., 2013; Kirchmair et al., 2015; Peyret and Krishnan, 2012; Sarigiannis et al., 2017
In vitro systems	In the absence of detailed information on the metabolism of a compound, it is pragmatic to use a metabolic system that contains a broad spectrum	Howgate et al 2006; Shiran

	of enzymes, such as hepatocytes or the S9 sub-cellular fraction with appropriate co-factor supplementation. If appropriate, other sub-cellular fractions, such as microsomes or cytosol, can be used to measure apparent *in vitro* intrinsic clearance. Techniques using recombinant enzymes with appropriate inter-system extrapolation factors (ISEF) or relative activity factors (RAFs) have also been used to measure apparent *in vitro* intrinsic clearance. Analogous approaches can be used to determine apparent intrinsic clearance in tissues other than the liver, if relevant for a particular chemical. Alternatively, an estimate of the apparent intrinsic clearance in each tissue can be obtained by using recombinant enzymes and accounting for differences in expression in the different organs.	et al 2006; Emoto & Iwasaki 2007; Gertz et al, 2011.
In vitro to in vivo extrapolation – scale up to organ	Various approaches have been suggested to attain accurate *in vivo* prediction of clearance from *in vitro* data. Understanding the particular *in vitro* system used and any bias or inherent under prediction is crucial for reliable extrapolation of clearance measurements. The apparent intrinsic clearance measurement determined *in vitro* should be corrected for non-specific binding in the *in vitro* system. Several approaches to determine or predict non-specific binding have been published.	Hallifax & Houston, 2019; Krause & Goss, 2018; Poulin & Haddad, 2018; Wood et al 2017; Hallifax & Houston 2012; Da-Silva et al 2018; Poulin & Haddad, 2013; Barr et al 2019; Nair et al 2016; Kilford et al 2008
Hepatic metabolism	Clearance rates or rate constants for metabolism in the liver can be derived from *in vitro* assays with liver S9 fractions or hepatocytes, and potentially from even more complex *in vitro* approaches (e.g. organ-on-a-chip; Annex 2). The more complex *in vitro* models offer the advantage of longer incubation times. If cell numbers are sufficiently high, this can lead to improved sensitivity and the determination of clearance rates for low clearance chemicals that are not measurable in standard assay systems. Care is needed when extrapolating the rate constants from the assay into a PBK model parameter. Depending on the structure of the model (one-box model for the whole organism, multi-compartment model with explicit blood flow, model with or without explicit mass transfer kinetics between the blood pool in the liver and the hepatocytes), different extrapolation approaches are needed.	A recent publication gives a systematic overview of all these possibilities and offers the respective extrapolation formulas. Krause & Goss, 2018
Biliary Clearance	Biliary clearance involves the transporter-mediated excretion of the unchanged chemical or its metabolite from the hepatocyte into the bile canaliculi. Some chemicals can be reabsorbed in the gastrointestinal tract, in a process known as enterohepatic recirculation (EHR). The same process can occur for metabolites (e.g. glucuronides), where a conversion back into the parent chemical can be mediated by the gut microbiome which is then reabsorbed in the colon. EHR has the effect of prolonging the exposure of the chemical in the body.,	
In vitro to in vivo extrapolation – scale up to organ	Biliary clearance may be defined as an overall intrinsic clearance term or J_{max}/K_m values obtained from sandwich cultured hepatocyte assays or recombinant expression systems respectively. Biliary intrinsic clearance	

	can be scaled by the number of hepatocytes per gram of liver to obtain organ-level biliary intrinsic clearance. For J_{max}/K_m values	
Renal Clearance	Renal clearance consists of glomerular filtration, passive and active reabsorption from the renal tubules back into the bloodstream and active secretion of the chemical from the blood into the renal tubules. As for hepatic clearance, there is not yet an accepted experimental method that reproduces all aspects of renal clearance in a single model. For some applications, if the chemical is metabolised extensively, it may be sufficient to ignore renal clearance for PBK modelling purposes as it may be a very minor elimination route for parent chemicals. However, for many chemicals, renal clearance can be predicted without the need for specific experimental data. For chemicals with low permeability, renal clearance can be estimated by a simple algorithm; the kidney filtration rate is the product of two parameters, the glomerular filtration rate (GFR) and the unbound fraction in plasma (*fup*). Mechanistic kidney models allow for more accurate estimates of renal clearance by accounting for filtration, secretion and reabsorption simultaneously in different segments of the nephron.	Varma, 2015; Cox, 2008; Tucker, 1981 See case studies 5 & 6.
Passive reabsorption	For chemicals with high permeability, passive reabsorption means that the chemical is likely to be reabsorbed into the circulating blood from the proximal tubule. Thus, a PBK model that does not account for passive reabsorption will over-predict the renal clearance rate and under-predict the systemic concentration. More complex models of renal clearance have been published or implemented in commercial software and can account for both passive reabsorption and active transport of chemicals. Kinetic parameters for passive reabsorption are usually obtained from uptake experiments with renal tubular cells, and subsequently scaled by surface area or number of cells per gram of kidney. If it is necessary to account for passive reabsorption, more complex models of renal clearance have been published or implemented in commercial software. Chemicals can also be actively reabsorbed from the proximal tubule or actively excreted via transporters found on the apical and basolateral membrane of renal tubular cells. These processes can influence the renal clearance of chemicals with low permeability.	Niederalt et al, 2013; Huang & Isoherranen 2018
Michaelis-Menten parameters for metabolite formation	Michaelis-Menten kinetics is the well-known approaches to enzyme kinetics in every organ of the body. Metabolism is usually described using V_{max} and K_m measured in *in vitro* systems can be informative. Up to date no *in silico* model can quantitative predict these parameters.	
***** These models often incorporate scaling of *in vitro* measured metabolic parameters to estimate the metabolic clearance *in vivo* (e.g. scaling up intrinsic clearance values determined in microsomes to whole liver). The basis for this scaling is addressed in previous international guidance on PBK modelling (WHO, 2010).		

Table 2.9. Clearance parameters – pointers for the modeller and assessor

Issue	***What to do****
Computational estimates of metabolic rate constants can be unreliable, especially if the PBK model output is highly sensitive to these parameters.	Measurement of metabolic rate should be performed using a suitable *in vitro* system and proper experimental setup, which should be reported.
Chemicals that exhibit high metabolic intrinsic clearances	Chemicals that exhibit high metabolic intrinsic clearances should not be immediately assumed to have high hepatic clearances, as this could be modified by active transport such as basolateral influx/efflux which would reduce hepatic uptake and amounts presented to metabolic enzymes.
Highly permeable chemicals	In the case of highly permeable chemicals, it may be necessary to account for passive reabsorption in the kidneys.
Compounds with poor passive permeability	It should not be assumed that compounds with poor passive permeability have renal clearances close to the glomerular filtration rate, as active transport can facilitate secretion or reabsorption, particularly for organic cations and anions, resulting in enhanced or diminished renal clearance respectively.
Individual active transport mechanisms may increase or decrease the rate of elimination, depending on the direction of transport.	Further investigate the biology and MoA of chemical, report the assumption when building the model
Chemicals that exhibit low metabolic intrinsic clearances	In chemicals that exhibit low metabolic intrinsic clearances require longer incubation times for a more reliable estimate of the clearance parameters. More complex *in vitro* models may be needed, uncertainties in the extrapolation of rate constants derived from such models into a PBK model parameter should be addressed.
Saturation kinetics	For saturable kinetics Vmax and Km parameter should be measured and introduced into a PBK model that considers the Michaelis Menten equation for metabolite formation. This is relevant for phase I and phase II metabolism.
*In cases where there is a residual uncertainty, which cannot reasonably be reduced through additional data generation, this uncertainty should be recorded. Wherever possible, an attempt should be made to qualitatively express the impact of this uncertainty, for example whether the assessment is expected to be more or less conservative as a result.	

2.5. Step 3 – Computer implementation (Solving the equations)

Many software packages are available with which the ordinary differential equations in customised PBK models can be numerically integrated and solved. A list of commonly used software is available in Madden et al. (2019) and in Annex 1. Integration algorithms that can be applied (e.g., Gear, Rosenbrock) need to be capable of handling "stiff"[1] sets of

differential equations (Rietjens et al., 2011). These numerical methods are well established, and are not considered to represent a significant source of uncertainty in the modelling process. Therefore, this guidance does not address this area further.

A certain degree of familiarity with the programming language used to code and solve custom PBK models is essential for end users who need to review the accuracy of computational implementation of PBK models. In cases where a commercial PBK platform is used, end users are not required to have programming skills or review for coding errors. However, EMA (2018) advises that a commercial PBK platform needs to be qualified for the intended purposes, which means demonstrating that the PBK platform can predict a specific outcome (e.g., competitive inhibition of an enzyme) for chemicals with similar ADME characteristics. FDA (2018) also advises including library system models of virtual population and library drug models with the submission of a PBK modelling analysis.

2.6. Step 5 – Model performance

2.6.1. Model output (Dose Metric)

Usually, the outputs of a PBK model are simulations that capture the time- and dose-concentration response curves of a chemical (parent or metabolite). As a time-concentration-response curve (Figure 2.3.A), the most common dose metrics used are the maximal peak concentration ($C_{max)}$ and the Area-Under-the-Curve (AUC).

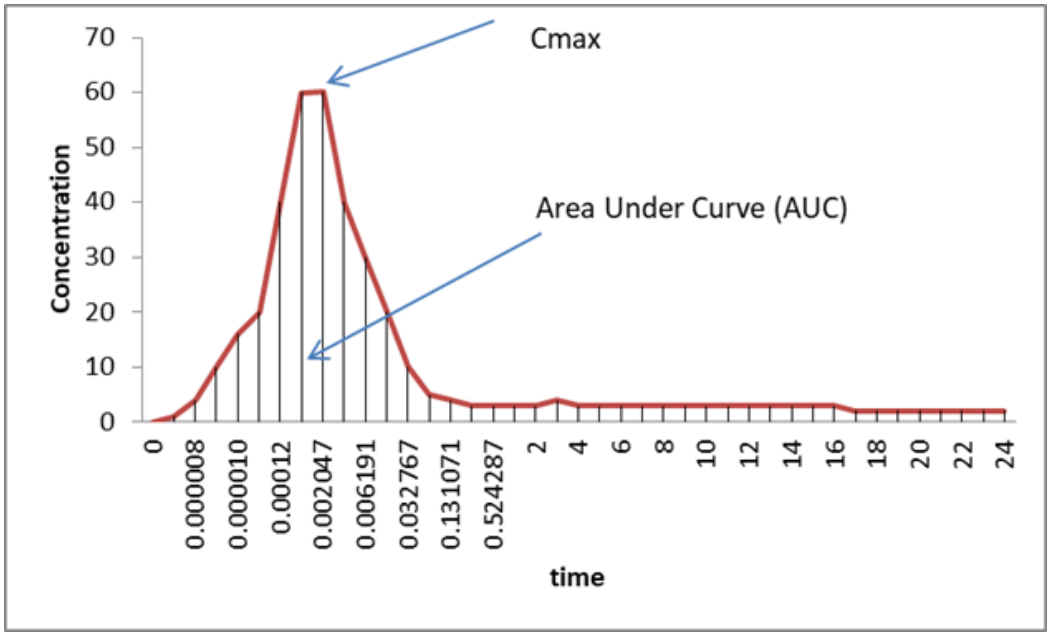
Achieving a dose-concentration response curve (figure 2.3.B) allows the extrapolation from external to internal and vice versa (reverse dosimetry). This will allow the user to achieve a specific dosimetry to apply together with *in vitro* data as point of departure.

The importance of understanding and quantifying inter-individual variability and the level of uncertainty in each step of a chemical safety assessment with non-animal methods is emphasised (Berggren et al., 2017). This requires the generation of probabilistic PBK models and the use of various stochastic sampling approaches such as Monte-Carlo sampling. Defining informative prior distributions around parameters converts a deterministic model to a probabilistic (population) model. Figure 2.4 reports an example of a Monte-Carlo simulation built by using *in vitro* data for a few individuals (for instance 22 human S9 fractions) and allowing the model to simulate for 10,000 individuals; the statistical distribution is addressed in more detail in Section 2.6.3.

PBK model outputs are usually validated by comparison against real (*in vivo*) data. Bessems et al., (2013) also proposed human microdosing as an approach to verify and validate PBK model predictions. However, when *in vivo* data are missing, alternatives are needed; the following chapters are oriented to give guidance on how to address this.

Figure 2.3: Examples, A. PBK model dose metric, time–concentration response curves (units are usually in days, hrs, or min for time and in in uM or ng/M etc. for concentration); B. Dose-concentration response curves (units are usually in mg/kg BW for dose while in in uM or ng/M etc. for concentration).

A.



B.

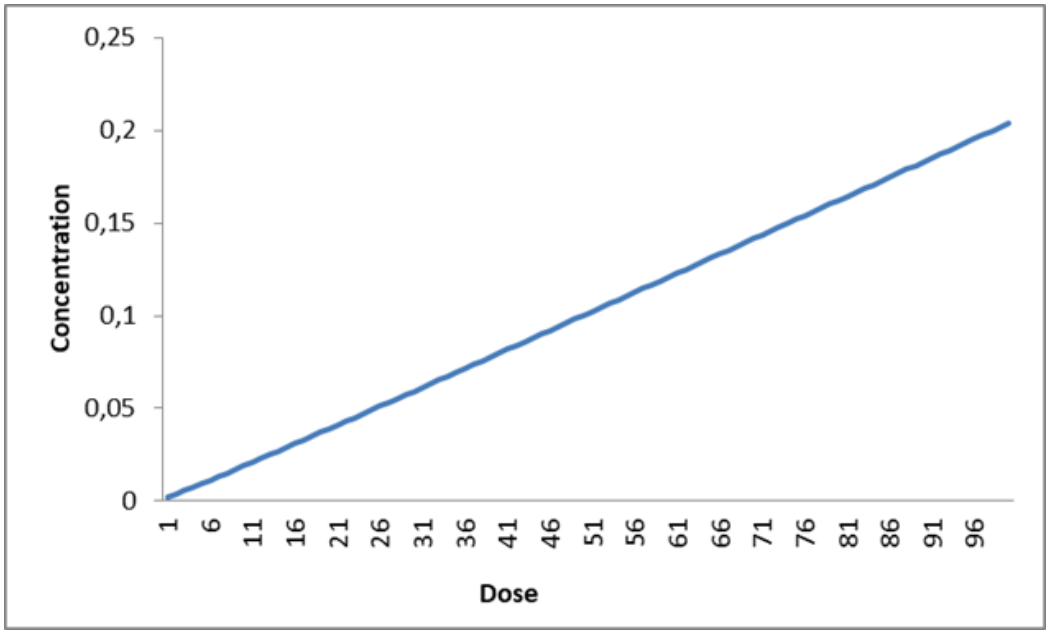
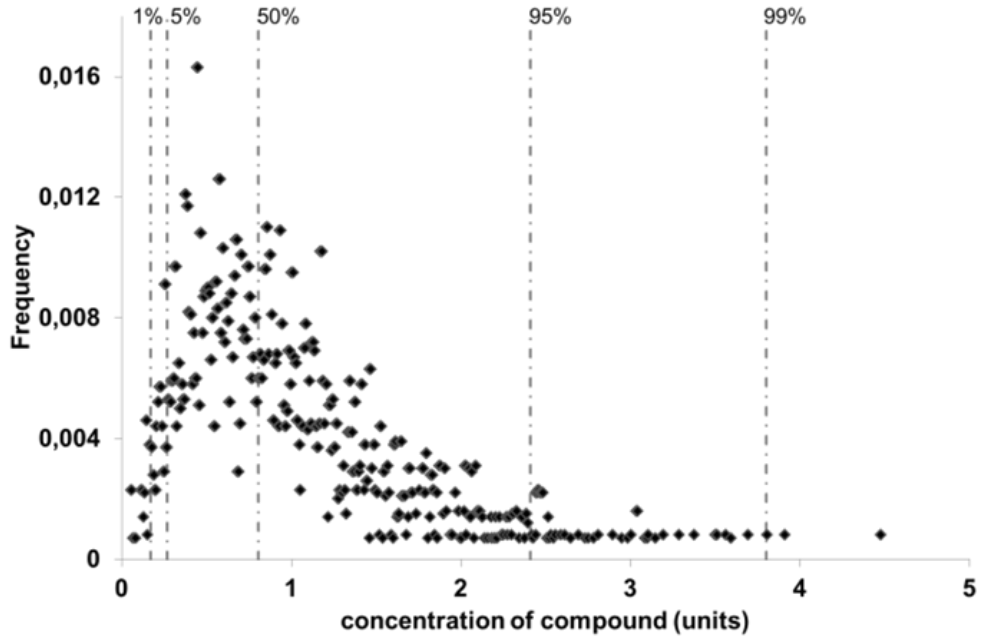


Figure 2.4 Example of a Monte-Carlo analysis of predicted frequency (10,000 simulations) distribution of a chemical levels for the general population based on the specific human individuals simulated at a certain dose. Highlighted with a vertical straight line several percentiles (see chapter 2.6.3).



2.6.2. Validation and uncertainty

The terms validation, uncertainty, sensitivity and variability are often used in different ways, which can lead to confusion and miscommunication, especially in the dialogue between modellers, experimentalists, and risk assessors/managers (see definitions in section 1.6).

As reported in section 1.6, the validation of a PBK model is the process of assessing the scientific validity of the mathematical model, based on five main characteristics: i) biological basis of the model structure and parameters; ii) theoretical basis of the model equations; iii) reliability of the input parameters; iv) sensitivity of model output to input parameters; and v) goodness-of-fit and predictivity of a given dose metric.

Analogous to the validation of a QSAR model (OECD, 2007) and the validation of new test methods (OECD GD 34, 2005), the attributes of PBK model validity are typically established by the model developer. In this sense, model validity is distinguishable from "applicability" or "adequacy", which depends on scientific validity, but also on additional contextual factors (see Chapter 3).

According to EFSA (2018), uncertainty is a general term referring to "all types of limitations in available knowledge that affect the range and probability of possible answers to an assessment question". There are many sources of uncertainty in a regulatory assessment, including those inherent to modelling approaches such as PBK models. This guidance addresses uncertainties in the assessment of PBK modelling for regulatory application; it does not address other types of uncertainties (e.g. limitations of existing hazard data) which are typically addressed in regulatory assessments/conclusions.

The uncertainty in the use of a PBK model can be divided into:

1. uncertainties in the model input parameters (i.e., principally, variability in the parameter estimates due to intrinsic biological variation or measurement error)
2. uncertainty in the model algorithm / structure (biological basis)
3. uncertainty in the model output(s), deriving from uncertainties in the input parameters and model structure

A distinction is sometimes made between "epistemic" uncertainty, resulting from incomplete knowledge of a system, and "aleatoric" uncertainty, which is an inherent feature of a system. The former type of uncertainty can, in principle, be reduced, e.g. through experimentation, while the latter can only be characterised, but not reduced. Often, when the word "uncertainty" is used, the former (more restrictive) sense is intended, whereas "variability" is more commonly used for aleatoric uncertainty.

In PBK models, model structure represents an epistemic uncertainty, while there may be one or both kinds of uncertainty in input parameters. Uncertainties related to natural variability (i.e. physiological differences occurring between individuals in a population, or between different points in time for a given individual, but can be considered in population simulations) are in principle irreducible – they do not have a single value. Conversely, uncertainties related to measurement error (experimental reproducibility and reliability) in the case of measured properties, or to calculation error (model reliability) in the case of predicted properties, can be reduced (up to a limit) – in principle, they generally have a single value, although in practice, measurements cannot be determined with absolute precision.

In uncertainty analysis, uncertainties are represented by (probability) distributions, irrespective of their origin. Generally, in PBK modelling, “uncertainty analysis” explicitly accounts for uncertainties (i.e. variability) in the input parameters; however, the impact of choosing alternative model structures is rarely included. It should also be noted that a model cannot take into account "unknown unknowns", which may influence the behaviour of a system.

2.6.3. Uncertainty (model parameters) and sensitivity analysis

Uncertainty and Sensitivity Analysis are two important techniques in the validation of a PBK model, and are typically carried out together. The aim of uncertainty analysis is to determine the overall uncertainty in model output, given the uncertainties in the model input parameters, whereas the aim of sensitivity analysis is to quantify how much of the overall uncertainty in the model output can be attributed to each input parameter.

Sensitivity analysis (SA) allows the uncertainty in model output to be ascribed to one or more input parameters within the model, thereby offering a means of ranking the parameters based on their relative contribution to the model output (Saltelli *et al.*, 2004).

When using experimental data to estimate model parameters, the question may arise as to whether all parameter estimates are unique. Assuming we have output data for a given experimental condition, identifiability analysis can be used to determine if there is a set of unique parameters that can describe the observed conditions. In general, there are two types of identifiability cases:

- *structural identifiability* is related to the model structure selected before performing the estimation.

- *parameter identifiability* is related to the ability to estimate unique parameters using experimental data which includes measurement error and variability *(irreducible uncertainty)* in model input parameters

There are many SA methods that exist for identifiability analysis application for pharmacokinetic models. Structural identifiability can be addressed with local sensitivity analysis. Also known as one-at-a-time (OAT) SA, this technique examines the predicted change in output/input for each unknown parameter. An established OAT calculation consists of the linearization of the sensitivity coefficients by using a normalized percent change (output/input) calculated per parameter. The absolute value of the normalized sensitivity coefficients can be used to rank the relative contribution for each model parameter and help determine if the parameters are identifiable. OAT has a role in structural identifiability and identification of subsets of non-identifiable parameters. The model structure containing non-identifiable parameters can be reformulated or simplified to account for the revised number of parameters. This "OAT" approach is most commonly used, but provides unrealistically small estimates of model uncertainty in most cases.

Practical identifiability has a wide range of available techniques that make use of confidence regions and statistical analysis. These include both OAT and global sensitivity analysis (GSA) techniques. An example of an OAT application makes use of the Jacobian or Fisher Information Matrix (FIM) methods developed for non-linear models. The FIM application looks at correlation between parameters while examining parameters sensitivity over time searching for non-identifiable zones (Bonvin et al. 2016; McLean and McAuley 2012).

GSA examines all parameters at the same time and is preferable when there may be substantial interaction between estimated model parameters (Campolongo & Saltelli, 1997; Campolongo et al., 1999; Loizou et al., 2008; Saltelli et al., 2000; Saltelli et al., 2004). In cases where model parameters are estimated from multiple methods (e.g., using different *in silico* models to predict values for the same parameters), plausible ranges of model parameters may be large and global SA should be conducted in these cases. McNally et al (2011) proposed a two-step approach for global SA (Campolongo & Saltelli 1997): 1) Morris test as a screening exercise followed by 2) the extended Fourier Amplitude Sensitivity Test (eFAST) for quantitative analysis.

The choice of OAT vs GSA will necessarily be determined by the complexity of the model, the available resources (including computing, time and personnel resources) and the context of use.

Both uncertainty and sensitivity analysis require parameter ranges derived from statistical distributions for the PBK model parameters. Anatomical and physiological parameter distributions can be obtained from the freely available web-based application PopGen, which is a virtual (healthy) human population generator (McNally et al., 2014) (http://xnet.hsl.gov.uk/popgen). For example, a human population, comprising 50% male and 50% female, black American, age range 16–65, height range 140–200 cm, body mass indices 18.5–30 could be generated to encompass the characteristics of a study population. In PopGen, organ masses and blood flows are determined for virtual individuals from a priori distributions of anthropometric parameters such as body mass, height and body mass index and measured data from published studies. The original algorithms were derived and evaluated by Willmann et al. (2007) and modified by McNally et al. (2014).

Parameter ranges can be set at the 5th and 95th percentiles of the distributions. When there are no available distributions for parameters such as *in silico* derived PCs and fraction

bound in plasma, uniform ranges may be set based on reasonable assumptions such as, the lower range set equal to the mean divided by two and the upper range set equal to the mean multiplied by two; these default ranges may be subsequently revised in light of results from uncertainty and sensitivity analysis. Table 3A in annex is an example of the presentation of model parameters for sensitivity and uncertainty analysis and other probabilistic modelling applications. The parameters are described along with the abbreviation used in the model code, the mean value and the distribution where, N, ln and U denote normal, lognormal and uniform distribution distributions.

2.6.4. Sensitivity Analysis Workflow

The workflow comprises the following steps:

1. Perform the screening exercise using the Morris Test
2. Identify and select the most important parameters
3. Identify the time period where model output variance is of interest
4. Perform eFAST on the most potentially explanatory subset of parameters
5. Present the main effects, total effects(S_{T_i}), and interactions(S_i) as a Lowry plot (Fig 2.6)

An example of the results from a Morris screening exercise is shown in Figure 2.5.

The sensitivity indices, μ and σ are plotted for all model parameters, usually 20+ depending on the size and complexity of the model. Typically, ten or fewer have a significant contribution to model output variance and may be ranked according to significance. In this example there are two clusters with eight annotated parameters in one cluster and the rest non-annotated parameters close to the origin. All eight parameters and two or three of the next ranked parameters are carried over for eFAST analysis. A typical example of the results of an eFAST analysis presented as a Lowry plot is shown in Figure 2.6.

Figure 2.5. Morris screening exercise for variance in the excretion of a metabolite in urine (Curine). Rate of urine flow (Rurine), creatinine concentration (Creat), Fraction metabolised (FracMetab), first-order urinary elimination rate constant (K1), Fraction of dose taken up (FracDOSE), microsomal protein yield (MPY) and body weight (BW) and *in vitro* clearance half-life (Minch_T½).

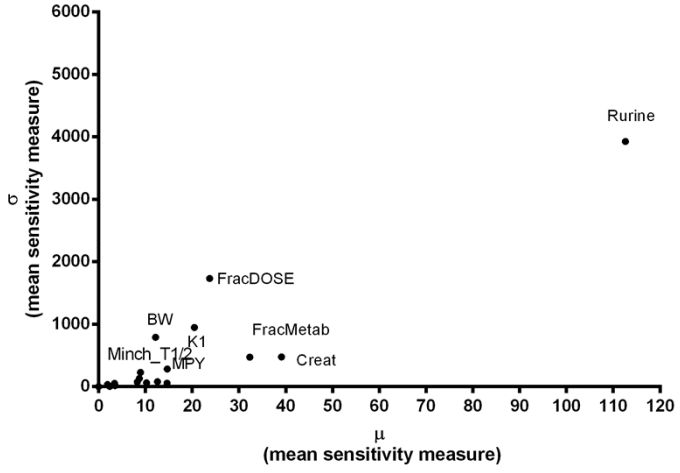
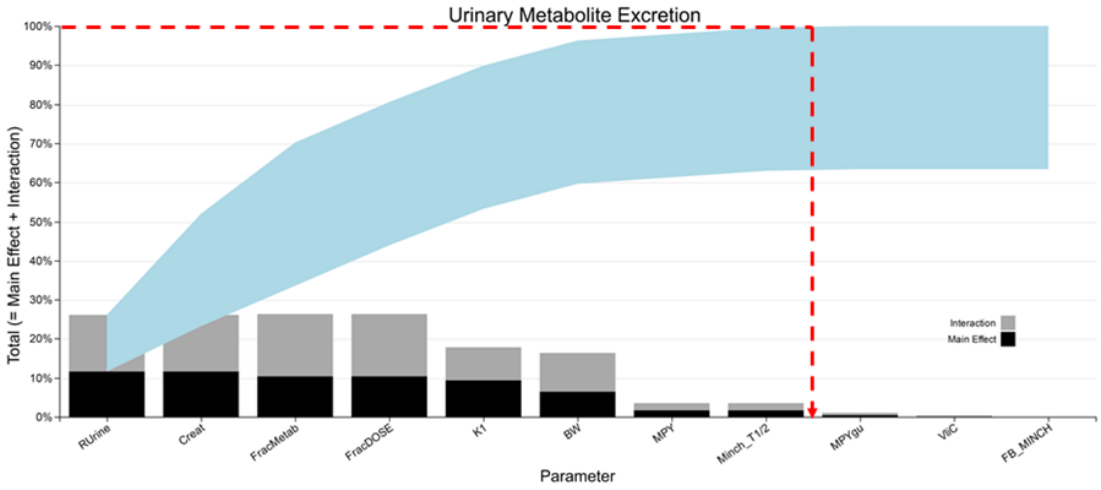


Figure 2.6. The Lowry Plot: Intuitive interpretation of global sensitivity analysis in this case of the eFAST quantitative measure.



Parameters are ranked from left to right according to the magnitude of Total effects (S_{Ti}) at any given time during a simulation (Figure 2.6). The total effect of a parameter is comprised the main effect (black bar) and any interactions with other parameters (grey bar) given as a proportion of variance. The ribbon, representing variance due to parameter interactions, is bounded by the cumulative sum of main effects (lower limiting line) and the minimum of the cumulative sum of the total effects (upper limiting line). The number of parameters that account for any given proportion of Total variance may be identified as shown with the broken line e.g., 100% by running a line from the y axis (Total=Main Effect + Interaction) to the ribbon then running a line down to the x-axis. Only those parameters to the left of that line have a significant contribution to Total variance.

2.6.5. Assessment of model predictive capacity by using a read-across approach

In the absence of *in vivo* kinetic data, one approach for establishing confidence in the predictive ability of a PBK model is to determine the 95% credible interval around the predicted kinetics for chemical analogues, for which *in vivo* kinetic data are available (see also chapter 3). In effect, the predictive ability of the model for one or more analogues ("source chemicals" for which biokinetic data are available) is used to demonstrate the applicability of the model to the chemical of interest ("target chemical") with similar ADME-relevant properties (but for which biokinetic data are lacking).

Analogues can be identified based on their structural, physicochemical and/or ADME properties. The availability of tools for identifying analogues, and characterising their properties, is described in several articles (Ellison et al., 2018; Madden et al, 2019; Patlewicz et al 2017; Pawar et al 2019).

The process for selecting suitable analogues, and choosing a suitable metric for judging similarity, is equivalent to the use of analogues for filling data gaps by read-across. Practical guidance on the use of analogues for data gap filling has been published by the OECD (2014) and various regulatory bodies (ECHA 2017). These documents typically provide stepwise workflows to support the process of grouping chemicals and reading across properties of interest from the analogue(s) to the target. In addition, an uncertainty

assessment framework for read-across has also been proposed (Schultz et al, 2019). Examples have been published in the literature (Schultz & Cronin, 2017) and as part of the OECD IATA Case Studies Project (OECD, 2017).

It is important to identify and characterise the same ADME-relevant properties for both the target and analogue, as the basis for the read-across argument (Ellison et al., 2018). Figure 2.7 provides a workflow that uses the principles of grouping and read-across when this is used in the context of data gap filling.

The workflow is based on 4 steps:

Step 1. Identification of analogues. Characterise the target chemical in relation to properties relevant to PBK modelling – physicochemical and ADME-related properties etc. Select appropriate software / QSARs to generate missing values or measure key *in vitro* parameters of analogues and where possible also generate data for the target chemical. Determine range of predictions (consider in relation to sensitivity analysis of resulting PBK model). With this information identify possible analogues.

Step 2. Selection and justification of analogues. Cross check that the identified analogues, have the information needed, biokinetics and/or existing valid PBK models (if sufficient date are available to generate a new PBK model for an analogue this can also be done). Assess analogues to select and justify inclusion or exclusion. Make a shortlist; take the most similar to the target chemical for further analysis, following the established criteria (Criterion 1. Chemicals with biokinetic data and similar ADME properties; Criterion 2. Chemicals with valid PBK model).

Step 3. Use of analogues in PBK modelling. Use analogue data to adapt or run the PBK model for the target chemical, substituting relevant parameters for the target chemical where possible.

Step 4. Reporting. Use reporting template provided in chapter 3 to register each decision taken.

The approach presented in Figure 2.7 refers to exposure to one chemical via one exposure route[2] (oral, inhalation, or topical/dermal) for different problem formulations (risk assessment questions). Additional uncertainties can be found when going route to route or cross-species. The selection of source chemicals can be guided by a scoring system and the information prerequisites (Paini et al., submitted).

Case studies 4 and 9 illustrate the use of the read-across approach in model validation with the alkenylbenzene family and caffeine respectively. Case study 8 shows route to route extrapolation for caffeine. Figure 1 in case study 8 shows how using PBK modelling approaches to predict animal, as well as human internal exposure dose metrics, provides an opportunity to investigate the consequences of variations in human dermal exposure scenarios; case study 8 is based on Bessems et al., 2017.

These case studies provide proof-of-concept. In practise, the ability to apply the read-across approach will depend on the availability of suitable analogues that are sufficiently data rich in terms of their kinetic properties.

2.7. Step 6 – Model Documentation (reporting)

An important step in establishing confidence in the application of a PBK model is to provide structured and adequate documentation. All information should be reported in order to help the model user; it should be reported and justified if data were omitted during

model development. As detailed in Chapter 3, model reporting should clearly describe the steps taken in the modelling, justify key assumptions, and provide key attributes of the model relating to its scientific validity. Additional information such as the availability of model code, and the extent of peer engagement, should also be reported.

2.8. Contextualisation of the PBK model for risk assessment

PBK models based on specifically generated *in vivo* data have long been applied in regulatory risk assessment to better characterise interspecies and intraspecies (interindividual) variation in kinetics, and to support high dose to low dose, and route to route extrapolations (EPA, 2006; WHO, 2010, example in table 1.1). Such models have gained increasing use, for example in the development of chemical specific adjustment factors (Bhat et al., 2017), and in the use of biomonitoring data to estimate (external) exposure levels (Verner et al. 2012; McNally et al. 2012).

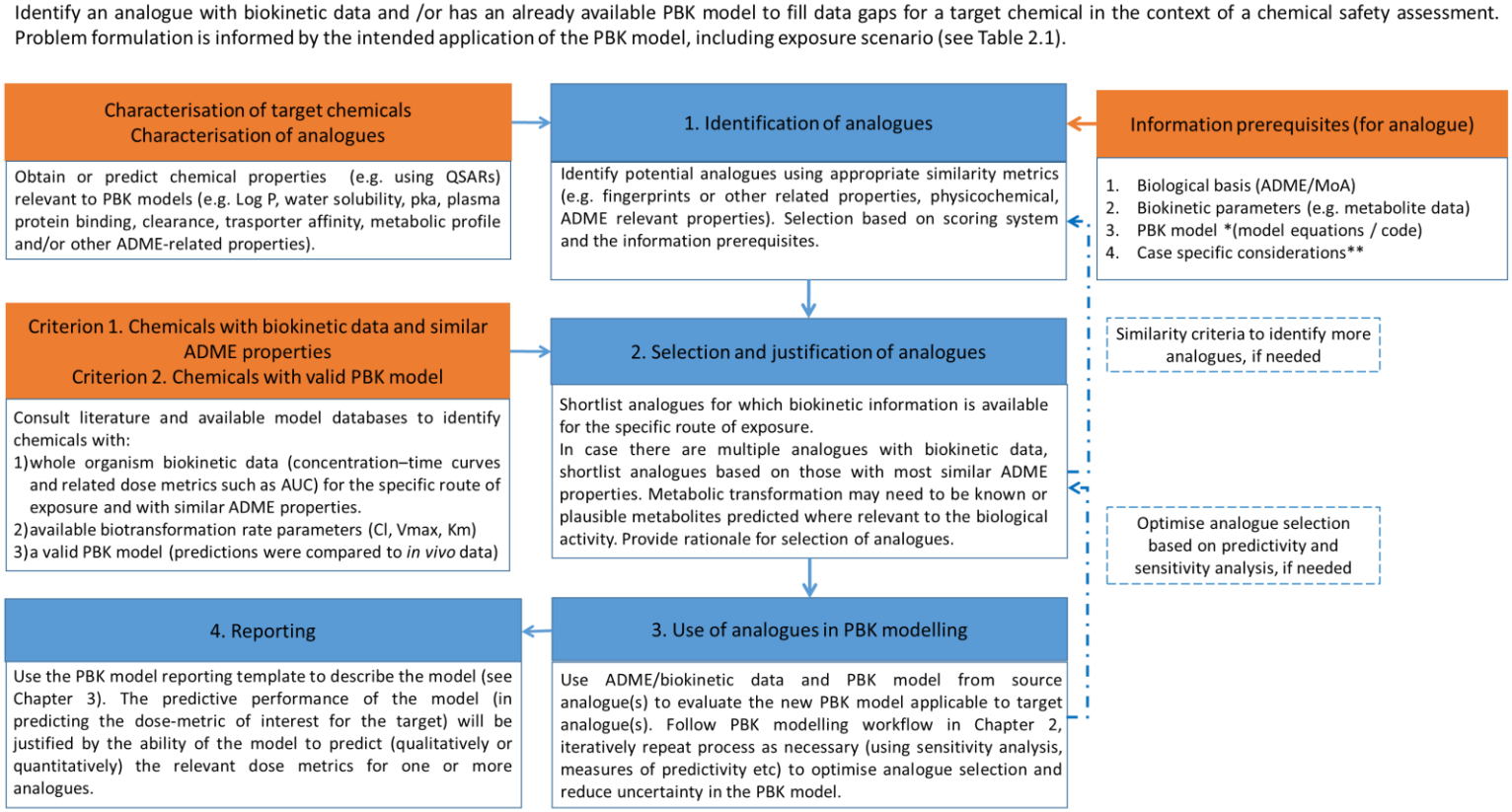
More recently, to reduce the reliance of chemical risk assessments on whole animal toxicity data, increasing emphasis is being placed on the use of *in vitro* and *in silico* approaches to link key molecular mechanisms with adverse outcomes. In this context, PBK models are a tool for refining extrapolations from animal toxicity testing results to human health risk estimates. Rather, PBK models are expected to play a critical role in converting *in vitro* concentrations that elicit cellular/sub-cellular responses (an *in vitro* Point of Departure, PoD) to the corresponding *in vivo* external doses that results in a simulated unbound concentration at the toxicological target site for comparing with the known exposure levels in a population (often referred to as quantitative *in vitro* to *in vivo* extrapolation or QIVIVE, see case study 3, *In vitro*-to *In vivo* extrapolation (IVIVE) by PBTK modelling and 7 Quantitative Proteomics-based Bottom-up PBK Modelling to Predict Chemical Exposure in Humans). Defining the proper *in vitro* point-of-departure at the cellular level and extrapolating it to an *in vivo* apical endpoint alterations, often occurring on a different time scale, is a challenging task (Zhang et al 2018), which PBK model can help in addressing. PBK models are also expected to play an increasingly important role in Integrated Approaches to Testing and Assessment (IATA)[3] to make better use of existing toxicity data and inform testing needs (Sachana, 2019). Thus, there is a need to establish consensus on approaches for characterising and validating PBK models for chemicals that do not have enough *in vivo* kinetic data for model calibration and validation. This situation can be regarded as extending the use of alternative methods for toxicity testing, in the sense that PBK models developed for these chemicals must rely on kinetic data generated by *in vitro* and/or *in silico* methods. An example of such use in assessment of chemical can be found in EMA (2020).

Thirteen case studies (listed in Annex 4) accompany this document as illustrative examples covering several applications in human and environmental risk assessment. These include:

- read across to model analogues (case study 4);
- environmental and human (bio)monitoring, internal species sensitivity distributions (case studies 1 & 2);
- interspecies differences extrapolation (case studies 5 & 6);
- inter individual differences extrapolation (case study 13);
- acute to chronic/high to low doses/ short and long term (animal vs human, occupational vs population) extrapolations (case study 11);

- route to route extrapolation (case study 8);
- quantitative *In vitro* to *in vivo* extrapolation, deriving a Point of Departure using QIVIVE and estimated free *in vitro* concentrations (case studies 3, 7, 10 & 13);
- next generation risk assessment of dermally applied consumer products (case study 12).

Figure 2.7. Schematic workflow to identify and use analogues in PBK model development and validation (Paini et al., submitted).



Notes

[1]A stiff equation is a differential equation for which certain numerical methods for solving the equation are numerically unstable, unless the step size is made extremely small.

[2] However, if a PBK model is available for a close analogue for oral route this PBK model could be used to predict for a target chemical via dermal route if the equations and the chemical and physiological parameters were adjusted accordingly. This would be a 2-step prediction (i.e. first adapt the PBK model to be used for the target chemical and then adapt it again for the change of route. This will generate probably more uncertainties which should be reported). A similar argument could be made for going cross-species.

[3] IATA are pragmatic, science-based approaches for chemical hazard characterisation and/or risk assessment that rely on an integrated analysis of existing data coupled with the targeted generation of data in tailored, often mechanistically-based, testing strategies, including *in vitro* data (OECD, 2016)

Chapter 3. Regulatory assessment of PBK models

Guidance documents are available from a range of national or supranational agencies addressing good practices in the documentation and assessment of PBK models for regulatory applications, including the evaluation of safety of drugs, industrial chemicals, food additives and contaminants and environmental toxicants (EPA 2006; EFSA 2014; CEN 2015; FDA 2018; EMA 2018) (See Introduction). Previous international guidance developed by the WHO drew significantly from source documents of several national agencies and reflected broad international experience (WHO, 2010).

This chapter builds on established principles, to additionally address the use of PBK models for non-traditional applications (e.g., converting *in vitro* point of departure to *in vivo* concentration) and to take account of situations where reliable *in vivo* kinetic data are lacking for model calibration/validation purposes. In these situations, the choice of "non-animal" methods used to parameterise the PBK model become critical when assessing the validity and applicability of the model. In this context, the use of sensitivity analysis (see also Chapter 2) is particularly important to determine the relative importance of parameters in driving the PBK model output(s), as a basis for considering confidence for purpose-specific application.

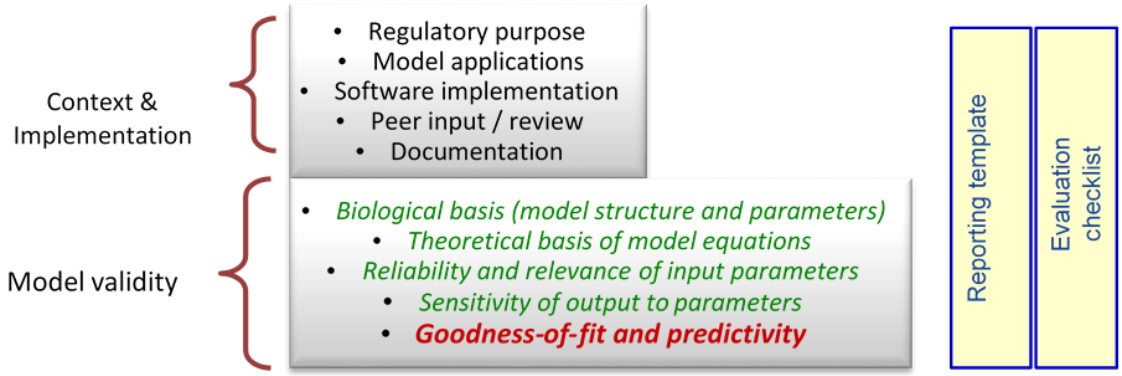
Accordingly, this chapter outlines a PBK Model Assessment Framework designed to address the following question, including for cases where reliable *in vivo* kinetic data are lacking: *is there sufficient confidence in the scientific basis of a PBK model to support its use in a specific regulatory assessment?*

The framework includes two categories of considerations in addressing the adequacy of a model for a given application (Figure 3.1). The first category is "Context & Implementation", which considers the regulatory application and context of use, which together determine the degree of acceptable uncertainty. Additional contextual factors include evidence of code availability and peer input/review, since these factors additionally influence the degree of confidence in a model.

The second category is "Model Validity", which addresses confidence and reliability in the model based on its intrinsic validation characteristics. Model validity consists of five main considerations, including four that do not require *in vivo* data (in green in Figure 3.1). The term "validation" is more broadly defined here than being limited, as is typically the case in the modelling community, to "goodness-of-fit and predictivity".

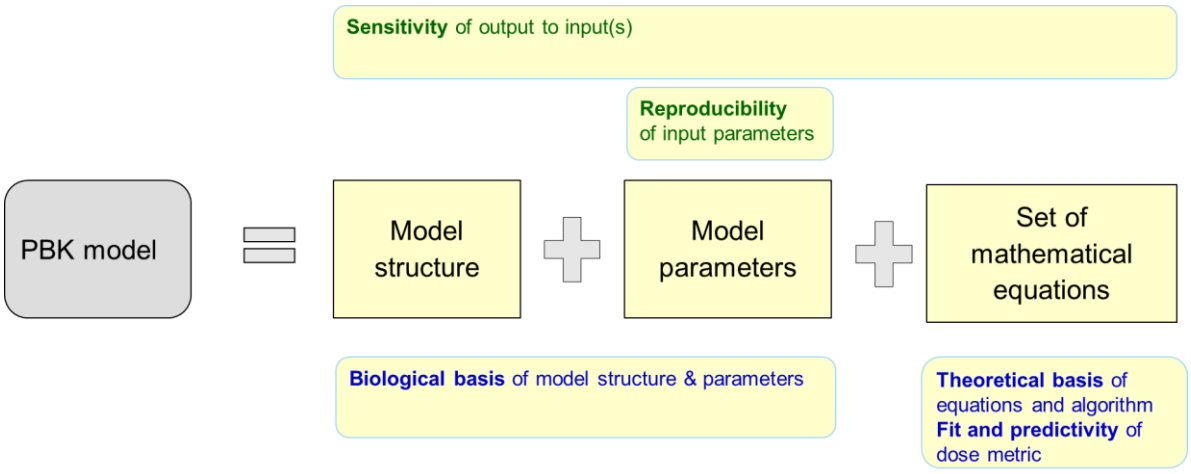
The level of confidence required for a PBK model will be dependent on the regulatory context of use. Such decisions lie outside of this assessment framework, and are determined by the regulatory assessor.

Figure 3.1. Characteristics of an Assessment Framework for PBK Models. Key information and supporting references are addressed in the model reporting template (by the model developer/proponent), while key questions to facilitate confidence assessment in the purpose-specific application of a model are outlined in the evaluation checklist (for use by the assessor/regulator). Considerations in green text do not require *in vivo* data; those in red require *in vivo* data.



Consistent with the OECD definition of validation (OECD 2005), the scientific validity of a PBK model can also be thought of in terms its relevance and reliability, as illustrated in Figure 3.2.

Figure 3.2. The scientific validity (reliability and relevance) of a PBK model. Reliability considerations in green (above), relevance consideration in blue (below).



The assessment framework comprises two tools, namely:

1. A "model reporting template" which prescribes the nature of adequate documentation of a PBK model to facilitate its independent evaluation by the regulatory/risk assessment community. This template is analogous to reporting formats developed in other areas of quantitative modelling such as QSAR (OECD, 2007). It is foreseen that the template will be compiled by the model developer to report PBK modelling analysis in the context of a specific regulatory assessment.

2. A "model evaluation checklist" to support the evaluation of relative confidence in the model by the regulator/risk assessor. The intended application and context of use determines the desired model characteristics.

These two tools are complementary but designed principally for different target audiences, though likely informative for both. The model-reporting template informs the modelling community about best practices in communicating models to support their application in a regulatory context. The associated evaluation checklist assists the risk assessment/regulatory community in considering whether the evidence supporting the use of a PBK model is sufficient for its intended application. In other words, the model-reporting template captures the evidence in support of a PBK model; while the checklist provides the basis for evaluating confidence in a specific use of the model, which is context dependent.

In addition to adequate description and evaluation to facilitate regulatory consideration/acceptance, continuing consultation of the risk assessment/regulatory community in model development to facilitate purpose specific application is critical, as is training of risk assessors and increased access of regulatory agencies to internal or external expertise in PBK modelling (Tan et al., 2018, Paini et al., 2019, WHO, 2010).

3.1. Context and Implementation

The intended application and use context of a PBK model in a regulatory assessment determines the required model capability and level of confidence in the model. In a PBK model submission, the specific question in risk assessment that the model addresses (e.g. cross-route or cross-species dosimetry, QIVIVE) should be clearly delineated, along with justification for the level of biological detail.

In addition to a schematic of the model structure, model developers should provide justification for the choice of model structure and dose metric. Description of the model structure delineates physiological compartments and biological processes relevant to the scientific question(s). The rationale for including specific physiological compartments or processes may also refer to models established for chemical analogues (see also Chapter 2.5.5). The selection of dose metric, as model outputs, should be based on hypothesised modes of action and associated weight of evidence, when possible. Specific points that the developer should address are framed as questions in the Evaluation Checklist below (sections A1-A2).

As a 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 and created following the required purpose/problem formulation. 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.

For custom PBK models, identification of the sources of model parameters should be provided with parameter names/symbols, meanings, values and units in a tabular format. Descriptions of *in vitro* and *in silico* methods used to estimate values of chemical-specific parameters should be provided along with justifications for selecting these methods (e.g. applicability domain for a QSAR model). Allometric scaling and *in vitro–in vivo* scaling of parameters should be described in text, shown in model equations, or referenced.

For custom PBK models, model code should be provided to ensure that:

- the model code (i.e. equations and parameter values) is devoid of syntax or mathematical errors.
- the values and units of input parameters are accurate.
- the chemical mass balance and blood flow balance are respected at all times; and
- there is no solver or numerical error.

For PBK platforms (e.g., Simcyp, Gastroplus, PKSim; see also Annex 1), the ability of the platform to perform the specific type of simulation(s) for the intended purpose(s) should be demonstrated by simulating compounds with similar ADME characteristics to that of the intended use. This process is called the qualification of a PBK platform (EMA, 2019). Evidence on the qualification of the platform, including input files for model simulations and output files that report the simulation results, should be provided.

Specific points that the developer should address are framed as questions in the Evaluation Checklist below (Table 3.2, section A3).

3.2. Model validity

The model reporting template makes reference to the five main considerations underlying model validity. One of these considerations, goodness-of-fit and predictivity, requires the availability of empirical *in vivo* kinetic data for comparison with PBK model predictions. The other four main considerations do not depend on the availability of *in vivo* kinetic data, namely the biological basis of model structure and parameters, the theoretical basis of model equations, the uncertainty in model inputs, and sensitivity of the model output to input parameters (Figure 3.1).

Sensitivity Analysis is recommended, as a means of identifying the model parameters that have a significant influence on model outputs. Since the overall uncertainty of model outputs is contributed by the uncertainty of these parameters (i.e., variability in the parameter estimates), special attention should be given to the generation of more reliable estimates for these parameters. SA provides a means of simulating the uncertainty in model outputs given a plausible model structure and plausible ranges of input parameters. SA thus can be used to evaluate confidence in the application of PBK models where parameters are estimated from *in silico* and *in vitro* data or based on those for analogue compounds. Application of OAT or GSA have a great impact on drawing conclusions on model validity. Further guidance on SA is given in Chapter 2 and Annex 3.

A table that may be helpful in considering uncertainty (including natural variability, reproducibility and reliability) of the input parameter estimates and sensitivity as a basis for characterising confidence and reliability in the model output is presented in Figure 3.3. The parameters requiring focus for evaluation are those with a high uncertainty and having a high influence on the model outcome. Conversely, the parameters requiring least evaluation are those with a low uncertainty and having a low influence on the model outcome.

Figure 3.3. Confidence matrix for the input parameters. Relative confidence in input parameters based on their uncertainty (natural variability, reproducibility, reliability) and their impact on the output of the PBK model (determined by sensitivity analysis). (WHO, 2010)

		Uncertainty in variability of the input parameter estimates		
		High	Medium	Low
SENSITIVITY	High		Parameter 1	Parameter 3 Parameter 4 Parameter 7
	Medium		Parameter 2	Parameter 10
	Low			Parameter 12 Parameter 13

Specific points that the developer should address are framed as questions in the Evaluation Checklist below (Table 3.2, sections B1-B5).

3.3. PBK Model Reporting Template

This model-reporting template delineates the nature of appropriate description of PBK models to support a broad range of regulatory applications and use contexts, including situations where *in vivo* kinetic data for comparison to assess model performance are limited or unavailable. While drawing upon and consistent with previous international guidance (WHO, 2010; FDA, 2018; EMA, 2018; Tan et al., 2020), the template includes additional considerations to address the expanding range and applicability of PBK models. The template provides a guide for model developers concerning aspects that should be reported to enable assessment based on the checklist in Section 3.5.

Table 3.1 PBK Model Reporting Template

PBK Model Reporting Template sections	Brief description of information to report for each section
A. Name of model	Provide a title of the model. The same should be reported in the checklist.
B. Model developer and contact details	Contact details of model developer.
C. Summary of model characterisation, development, validation, and regulatory applicability	Please capture main points in a brief summary regarding the development, validation and regulatory application.
D. Model characterisation (modelling workflow) Step 1 – Scope and purpose of the model (problem formulation) Step 2 – Model conceptualisation (model structure, mathematical representation) Step 3 – Model parameterisation (parameter estimation and analysis) Step 4 – Computer implementation (solving the equations) Step 5 – Model Performance Step 6 – Model Documentation	Follow the 6 steps of the modelling workflow chapter two. Report in detail the model structure, model biologically plausibility, and parameters with assumptions and limitations, tables can be placed under section H. parameter tables. Under model performance report information on sensitivity analysis, predictive performance. Strategy on how the model validation was performed, e.g. using analogues or other sources or approaches should be reported in detail.
E. Identification of uncertainties model structure input parameters model output other uncertainties (e.g. model developed for different substance and/or purpose)	For each step of the modelling workflow uncertainties should be reported. Use the information provided in the guidance to report and assess (e.g. table in figure 3.3. to capture information on sensitivity and uncertainty for input parameters).
F. Model implementation details software (version no) availability of code software verification / qualification	Information on the model equation solver/software to run the equation should be reported here.
G. Peer engagement (input/review)	Report the extent of peer engagement and review in development of the model.
H. Parameter tables	All information relevant to model parameterisation should be included here: physiological anatomical, physicochemical and biochemical. Report values and units and the source of the parameters (e.g. in case of *in vitro* studies detailed experimental conditions and motivation for choice of experimental conditions in case of non-guideline studies, in case of *in silico* studies add information on models).
References and background information publications links to other resources	Main reference and publications linked to development and description of the model

3.4. Checklist for Evaluation of Model Applicability

The overall level of confidence in a particular use of a PBK model depends on both contextual considerations and model validity (Figure 3.1). Relative confidence is addressed based on consideration of all these aspects, taking into account the intended application and context of use, including whether there are options for alternative assessment approaches without the PBK model.

This guidance does not stipulate how these considerations should be weighted, since this is expected to be context-dependent and determined by individual regulatory bodies. Instead, the focus is on describing an overall assessment framework that can be used to inform regulatory decision making. In particular, the evaluation checklist (Table 3.2) comprises a series of questions that can be used by the assessor/regulator to analyse the evidence provided by the model developer/proponent in the reporting template (Table 3.1).

As explained above, there are five main considerations underlying model validity. Considering goodness-of-fit and predictivity requires the availability of empirical *in vivo* kinetic data for comparison to model predictions. In cases where relevant and reliable empirical *in vivo* data, either for the substance of interest or for one or more analogues, are lacking, the decision to accept or reject a PBK model for the intended application will depend on the assessment of other four considerations. In such a situation, the use of GSA coupled with an assessment of the relevance and reliability of input parameters becomes particularly important. In addition to helping a risk assessor/regulator determine whether a model meets the minimum requirement for the intended application, the evaluation checklist (Table 3.2) can also be used to identify specific data/information needed to refine a PBK model before it can be accepted, or reasons for rejecting a PBK model submission.

A template for ascribing relative confidence in a PBK model for a specific application is included in Figure 3.4. The confidence in a PBK model is considered high if: 1) its structure and parameters have reasonable biological basis (biological basis); 2) the model has been tested against biokinetic (TK/PK) data in the species of interest and/or using analogues (model simulations of data predictivity); and 3) the uncertainty of the predicted dose metric(s) has been established based on SA (Uncertainty in input parameters and model output; Sensitivity of model output to input parameters). The rationale for confidence determination should be included in accompanying text (for examples see the case studies).

Figure 3.4. An illustrative scale of confidence levels for a PBK model (modified from WHO, 2010)

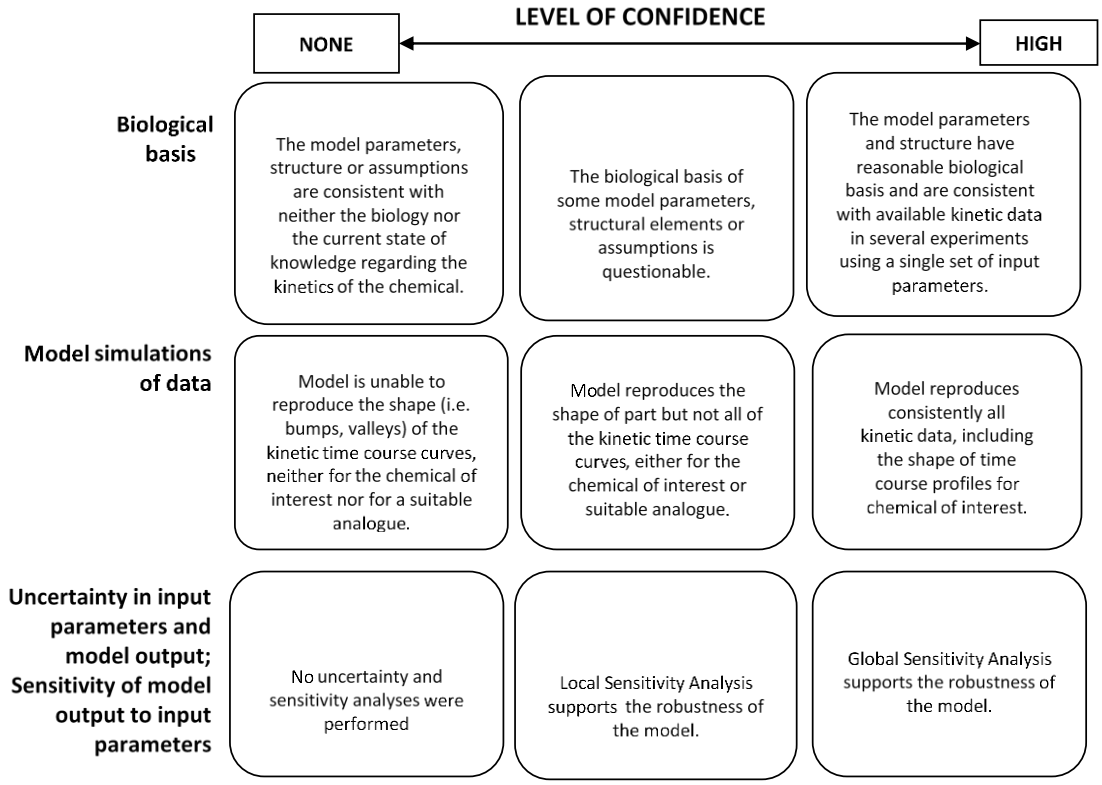


Table 3.2 PBK Model Evaluation Checklist

PBK Model Evaluation Checklist	Checklist assessment	Comments
Name of the PBK model (as in the reporting template)		
Model developer and contact details		
Name of person reviewing and contact details		
Date of checklist assessment		
A. Context/Implementation		
A.1. Regulatory Purpose		
1. What is the acceptable degree of confidence/uncertainty (e.g. high, medium or low) for the envisaged application (e.g. priority setting, screening, full assessment?)		
2. Is the degree of confidence/uncertainty in application of the PBK model for the envisaged purpose greater or less than that for other assessment options (e.g. reliance on PBK model and *in vitro* data vs. no experimental data)?		
A.2. Documentation		
3. Is the model documentation adequate, i.e. does it address the essential content of model reporting template, including the following:		
• Clear indication of the chemical, or chemicals, to which the model is applicable?		
• Is the model being applied for the same scientific purpose as it was developed, or has it been repurposed for a different application?		
• Model assumptions?		
• Graphical representation of the proposed mode of action, if known?		
• Graphical representation of the conceptual model?		
• Supporting tabulation for parameters (names, meanings, values, mean and standard deviations, units and sources)?		
• Relevance and reliability of model parameters?		
• Uncertainty and sensitivity analysis?		
• Mathematical equations available?		
• PBK model code available?		
• Software algorithm to run the PBK model code reported?		
• Qualification of PBK software platform?		
A.3 Software Implementation and Verification		
4. Does the model code express the mathematical model?		
5. Is the model code devoid of syntactic and mathematical errors?		
6. Are the units of input and output parameters correct?		
7. Is the chemical mass balance respected at all times?		

8. Is the cardiac output equal to the sum of blood flow rates to the tissue compartments?		
9. Is the sum total of tissue volumes equal to total body volume?		
10. Is the mathematical solver a well-established algorithm?		
11. Does the mathematical solver converge on a solution without numerical error?		
12. Has the PBK modelling platform been verified (for a different use, for instance, in the pharmaceutical domain)?		
A.4 Peer engagement (input/review)		
13. Has the model been used previously for a regulatory purpose?		
• Is prior peer engagement in the development and review of the model sufficient to support the envisaged application?		
• Is additional review required? Peer engagement includes input/review by experts on specific aspects of model development, individual reviews of the model by experts, or collective reviews by peer review panels. Availability of the comments and tracking of revisions to the model in response to peer input contributes to increased confidence in the model for potential application.		
B. Assessment of Model Validity		
B.1 Biological Basis (Model Structure and Parameters)		
14. Is the model consistent with known biology?		
• Is the biological basis for the model structure provided?		
• Is the complexity of the model structure appropriate to address the regulatory application?		
• Are assumptions concerning the model structure and parameters clearly stated and justified?		
• Is the choice of values for physiological parameters justified?		
• Is the choice of methods used to estimate chemical-specific ADME parameters justified?		
• Saturable kinetics processes (e.g. using Km and Vmax for metabolism).		
B.2 Theoretical Basis of Model Equations		
15. Are the underlying equations based on established theories,.e.g. Michaelis-Menten kinetics, Fick's laws of diffusion?		
• In the case of PBK models for particles, does the model take into consideration the properties of particles, e.g. particle size ranges, (poor) solubility, aggregation, partitioning and diffusion/sedimentation behaviour?		
B.3. Reliability of input parameters		

16. Has the uncertainty (individual variability, experimental reproducibility and reliability, effect of test conditions on the outcome of the study) in the input parameters been characterised?		
B.4. Uncertainty and Sensitivity Analysis		
17. Has the impact of uncertainty (individual variability, experimental reproducibility and reliability) in the parameters on the chosen dose metric been estimated?		
• Local sensitivity analysis?		
• Global sensitivity analysis?		
18. Is confidence in influential input parameter estimates (i.e., based on comparison of uncertainty and sensitivity) reasonable (within expected values; similar to those of analogues) in view of the intended application?		
B.5. Goodness-of-Fit and Predictivity		
19. For PBK models for which there are not sufficient *in vivo* data for the chemical of interest:		
• Suitability as analogue (chemical and biological similarity) been assessed?		
• Reliable estimation of chosen dose metric for analogue?		
• In general is the biological Variability of *in vivo* reference data (from analogue) established?		

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Glossary

Term	Definition / Explanation
Adverse effect	A change in the morphology, physiology, growth, development, reproduction, or, life span of an organism, system, or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress, or an increase in susceptibility to other influences.
Adverse Outcome Pathway (AOP)	An AOP is an analytical construct that describes a sequential chain of causally linked events at different levels of biological organisation that lead to an adverse health or ecotoxicological effect.
Alternative method	A method that replaces, reduces or refines the use of animals in toxicity testing
Analogue approach	Read-across between a small number of structurally similar substances; there is no observable trend or regular pattern in the properties.
Apical Endpoint	An observable outcome in a whole organism, such as a clinical sign or pathological state that is indicative of a disease state that can result from exposure to a toxicant. As such, the apical endpoint is representing a measurable outcome responding to multiple different toxicity pathways/MoAs and can potentially be indicative of adverse effects.
Calibration of a PBK model	During model development, the process of adjusting the model parameters to optimise the fit between the model output and existing (kinetic) data.
Dose-response relationship	The dose–response relationship describes the change (in nature, incidence, magnitude and/or severity) in an effect on an organism caused by different levels of exposure (or doses) to a stressor (usually a chemical) after certain exposure duration.
Characterisation of a PBK model	The process of establishing and transparently describing its attributes, including model structure and input parameters, details of the model building process, and other contextual factors, that are relevant for evaluating its suitability (adequacy) for a purpose-specific application. The important characteristics/attributes of a PBK model to support its regulatory use should be documented, usually by the model developer, using a reporting template (see 2.6 and chapter 3).
Category approach	Read-across between multiple substances that have structural similarity; there is an observable trend or regular pattern in the properties.

Term	Definition / Explanation
Confidence in the model or platform	a relative measure of the extent of trust in a model to adequately simulate the scenario of interest. This depends on the purpose-specific assessment of the model taking into account factors such as context of use and accepted range of uncertainty.
Context of use	The purpose (e.g. possible regulatory application/possible regulatory use) for which the model is being applied, including the metric(s) being predicted, the exposure scenario, and contextual factors such as regulatory options to request/generate additional data, or perform the assessment by other means.
Custom modelling software	Free or commercial software that is used for coding and solving a custom-built PBK model.
Group of substances	Substances that have physicochemical, toxicological and ecotoxicological properties that are likely to be similar or follow a regular pattern as a result of structural similarity may be considered as a group, or 'category' of substances.
In silico model	The technique of performing experiments via computer simulations. Examples include Structure-Activity Relationships (SAR) and Quantitative Structure-Activity Relationships (QSAR).
In vitro test	The technique of performing a given experiment in a test tube, or, more generally, in a controlled environment outside of a living organism.
Integrated Approach to Testing and Assessment	A structured approach used for hazard identification (potential), hazard characterisation (potency) and/or safety assessment (potential/potency and exposure) of a chemical or group of chemicals, which strategically integrates and weights all relevant data to inform regulatory decision regarding potential hazard and/or risk and/or the need for further targeted and therefore minimal testing.
Limit of Detection (LOD)	Lowest quantity (or a concentration) of a substance that can be detected (measured) in a sample using an analytical method.
Mechanism of action	A detailed molecular description of the mechanistic interaction through which a substance/molecule produces its effect.
Mode of action (MoA)	A biologically plausible sequence of key events at different levels of biological organisation, starting with the exposure to a chemical and leading to an observed (adverse) effect.
New Approach Methodology (NAM)	A recently coined term referring to any technology, methodology or combination thereof, that can be used to provide information on chemical hazard and risk assessment that avoids the use of intact animals.
Non-animal method	An alternative method that avoids testing in intact animals. The more traditional phrase for New Approach Methodology
PBK (PBBK, PBPK, PBTK) model	A mathematical model (set of equations) that describes the absorption, distribution, metabolism and excretion of a chemical in a given organism (e.g. human, fish, cattle) under a given exposure scenario. The model can be depicted in a conceptual diagram illustrating the route(s) of exposure (uptake) and excretion, tissue compartments (each represented with a unique set of physiological and physicochemical parameters), and processes

Term	Definition / Explanation
	occurring within (e.g. metabolism) and between (mass transfer) the compartments. Throughout this document we apply the more general term PBK model or modelling, noting that PBK, PBPK, PBBK and PBTK are synonyms. Physiologically based pharmacokinetic (PBPK) is the most widely used term for kinetic models describing the absorption, distribution, metabolism and excretion of a drug within the body. Although widely used in the pharmaceutical sector, the "PBPK" term is not strictly correct in the area of chemical risk assessment. An alternative is "PBTK" with the TK representing toxicokinetic, but this is not appropriate either (Clewell & Clewell, 2008). More general terms, such as physiologically based biokinetic (PBBK) or physiologically based kinetic (PBK), are thus more appropriate.
PBK model code	The implementation of a PBK model in a programming language for solving the PBK model equations.
Programming language	A set of commands, instructions, and syntax used to write code that implements a PBK model.
Purpose specific evaluation	Consideration of the uncertainties in the context of application – different levels of uncertainty are acceptable, depending on the application (context of use)
QSAR	Structure-Activity Relationship (SAR) is an approach designed to find relationships between chemical structure (or structural-related properties) and biological activity (or target property) of studied compounds. Qualitative relationships are derived from non-continuous data (e.g., yes or no data), while quantitative relationships are derived for continuous data (e.g., toxic potency data). Qualitative SARs and quantitative SARs, collectively are referred to as (Q)SARs.
Qualification of a software tool / platform	The process of establishing confidence in a commercial PBK platform to simulate a certain scenario, in a specific context, on the basis of scientific principles and ability to predict a large dataset of independent data thereby showing the utility of the platform. Qualification is thus purpose and platform version specific (EMA, 2019).
Read across:	A technique for predicting endpoint information for one substance (target substance), by using data from the same endpoint from (an)other substance(s), (source substance(s))
Sensitivity:	The sensitivity of a model parameter refers to the relative importance of different model input parameters in determining the model output. Thus, sensitivity analysis answers the question "for which of the input parameters was variability greatest?" (see 2.6.2).

Term	Definition / Explanation
Uncertainty	Uncertainty refers to all types of limitations in the knowledge available to assessors at the time an assessment is conducted and within the time and resources agreed for the assessment. A lack of precise knowledge about the model structure and/or the variability of the numerical value of a model input parameter (due to intrinsic biological variability or measurement error or inaccuracy in the *in silico* prediction of a model input parameter). More on model uncertainty is reported in 2.6.1.
Validation	For the purpose of this GD, the validation of a PBK model refers to the process of assessing the scientific validity of the mathematical model, based on five main characteristics: i) biological basis of the model structure and parameters; ii) theoretical basis of the model equations; iii) reliability of the input parameters; iv) sensitivity of model output to input parameters; and v) goodness-of-fit and predictivity of a given dose metric. *This definition is broader that the one typically used by model developers, which is often limited to the assessment of predictivity by comparing predictions with independent data (not used to train the model).
Verification of a PBK model:	The process of checking that the mathematical equation(s) are correctly implemented in the chosen software / platform.
Weight of evidence (WoE)	A stepwise process/approach of collecting and weighing evidence to reach a conclusion on a particular problem formulation including assessment of the degree of confidence.

Annexes

1) List of resources for PBK modelling

2) Prospective use of microphysiological systems in PBK models

3) Sensitivity analysis details

4) List of available Case studies

Annex 1. List of resources for PBK modelling

Disclaimer: The following tables are not necessarily exhaustive of all available resources, and no endorsement is implied. Tables are based on Madden et al. 2019, but expanded to include also environmental databases.

Table 1A. Physicochemical properties

Resource	Available from	Properties / Information
ACD /Percepta (ACD Labs)	https://www.acdlabs.com/products/percepta/	Log P; log D; pKa; Abraham solvation parameters (relating to hydrogen bonding ability, polarizability, volume and partitioning)[P]
ADME SARfari (EMBL-EBI)	https://www.ebi.ac.uk/chembl/admesarfari/	Log P; log D (reports values from ACD); PSA; [M,P]
ADMETlab	http://admet.scbdd.com/calcpre/index/	Log P; log D; log S
ADMET Predictor (SimulationsPlus)	https://www.simulations-plus.com/software/admetpredictor/	Log P; log D; pKa; diffusion coefficient; air:water partition coefficient; pH dependent solubility; solubility in gastric/intestinal fluid (fed and fasted states)
ALOGPS 2.1 (Virtual Computational Chemistry Laboratory)	http://www.vcclab.org/lab/alogps/	Log P; log D; water solubility; pKa [P]
Biobyte (Bio-Loom)	http://www.biobyte.com/	Log P, log D, pKa[P,M]
ChemIDPlus Advanced	https://chem.nlm.nih.gov/chemidplus/	Log P, pKa, solubility, vapour pressure, m.pt[M,P]
Chemspider (Royal Society of Chemistry)	http://www.chemspider.com/	Log P; water solubility pKa, vapour pressure, Henry's law constant[M,P]
ChemAxon	https://chemaxon.com/	Log P; log D; hydrophilic:lipophilic balance; water solubility; hydrogen bond donor / acceptor; pKa[P]

Corina Symphony (MN-AM)	https://www.mn-am.com/	Log P; hydrogen bond donor / acceptor parameters[P]
Computational Toxicology Dashboard	https://comptox.epa.gov/dashboard	Log P, m. pt, b. pt, vapour pressure, etc[M,P]
Episuite (US-EPA))	https://www.epa.gov/tsca-screening-tools/epi-suitetm-estimation-program-interface	Log P; water solubility, vapour pressure, Henry's law constant[M,P]
MOE (Molecular Modelling Environment)	https://www.chemcomp.com/MOE-Molecular_Operating_Environment.htm	Calculates >400 molecular descriptors including physicochemical properties, Topological Polar Surface Area (TPSA), log P, log D, pKa, electronic effects such as hydrogen bonding capacity, partial charges, dipole moment etc
Moka Molecular Discovery	http://www.moldiscovery.com/software/moka/	pKa[P]
Molinspiration	http://www.molinspiration.com/	Log P; hydrogen bond donors / acceptors; TPSA; volume
OECD QSAR toolbox	https://www.qsartoolbox.org/	Multiple physicochemical properties[M,P]
PubChem Open Chemistry database	https://pubchem.ncbi.nlm.nih.gov/search/	Multiple physicochemical properties including log P, TPSA, water solubility, pKa, vapour pressure[M,P]
Schrodinger: EPIK QikProp	https://www.schrodinger.com/	pKa; Log P; water solubility
SwissADME	http://www.swissadme.ch/	Multiple physicochemical properties including log P (various methods of calculation), water solubility, TPSA; no. hydrogen bond donors / acceptors[P]
(Bitterman et al, 2014, 2016).	(Bitterman et al, 2014, 2016).	membrane-water partition coefficients for neutral and ionic chemicals

Table 1B. ADME parameterisation tools/databases

Resource	Available from	Properties / information
ACD/Percepta	ACD Labs https://www.acdlabs.com/products/percepta/	Estimates multiple ADME-PK related parameters including absorption, bioavailability, Cp (T), Tmax and Cp (max), AUC, Pgp substrate specificity, Vd, protein binding. Blood Brain Barrier (BBB) penetration etc.
ADME database; Fujitsu	http://www.fqs.pl/en/chemistry/products/adme-db/	Interactions of substances with Phase I and II metabolising enzymes and drug transporters; database of kinetic parameters – *in vitro* assay model (Km, Vmax, Ki, Ks, efficiency, IC50, EC50, t½ etc)
ADMETlab	http://admet.scbdd.com/calcpre/index/	Human intestinal absorption; Caco-2 permeability; P-gp / CYP substrates and inhibitors; bioavailability; plasma protein binding; BBB partitioning; volume of distribution; t½ , clearance
ADMET Predictor (SimulationsPlus)	https://www.simulations-plus.com/software/admetpredictor/	Permeability (skin, cornea, gasto-intestinal tract, BBB); interactions with OATP1B1 and P-gP; plasma protein binding; blood:plasma ratio, volume of distribution; fraction unbound in microsomes etc)
admetSAR	http://lmmd.ecust.edu.cn/admetsar1/	Dataset for ADMET properties curated from literature; ADMET-Simulator also predicts approx. 50 relevant ADMET endpoints. (Human intestinal absorption, bioavailability, volume of distribution, plasma protein binding, clearance, Ki IC50 etc)
ADME SARfari (EMBL-EBI)	https://www.ebi.ac.uk/chembl/admesarfari/	Identifies ADME targets; finds pharmacokinetic data for input chemical or similar compounds
ADMETNet	http://bioinf.xmu.edu.cn/ADMETNet/index.html	Depicts pharmacokinetic pathways for drugs; provides data such as half-life, free fraction in plasma bioavailability, volume of distribution etc
ARC fish	https://arnotresearch.com/databases/	Fish whole-body *in vivo* biotransformation rate
ARC fish dietary	https://arnotresearch.com/databases/	Fish dietary bioaccumulation and toxicokinetics
BIOVIA Metabolite: Biovia (formerly Accelrys)	http://accelrys.com/products/collaborative-science/databases/	Compilation of *in vitro* and *in vivo* metabolic data from literature, conference proceedings and New Drug Applications
Brenda	http://www.brenda-enzymes.org/index.php/	Extensive database of Vmax, Km, Kcat and other parameters related to enzyme kinetics.
Computational Toxicology Dashboard	https://comptox.epa.gov/dashboard	ADME data to be included in this database (ongoing)
Cytochrome P450 Drug Interaction Table	https://drug-interactions.medicine.iu.edu/MainTable.aspx	List of drugs acting as substrates, inhibitors (partial ranking as to weak, moderate or strong) and inducers of CYP enzymes - 1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1 and 3A4,5,7

DIDB - Metabolism and Transport Drug Interaction Database	https://www.druginteractioninfo.org/	*In vitro* and *in vivo* drug interaction data from literature and New Drug Applications (NDA)
Drugbank	https://www.drugbank.ca/	Key ADME properties for drugs e.g. % oral absorption, volume of distribution, protein binding, metabolic information, t ½ , clearance etc
Drug Metabolism and pharmacokinetics Analysis Platform (DruMAP)	https://drumap.nibiohn.go.jp/	An online tool for the prediction of fraction unbound (fu,p value, fu, brain value), fraction absorbed (Fa), P_{app} (permeability coefficient for Caco-2), and D-Sol (solubility near pH 7 by dried DMSO method).
e-PK gene	https://www.druginteractioninfo.org/	Information on the impact of genetic variation on parent compound pharmacokinetics (i.e changes in AUC, Cl or Cmax for different populations)
EDETOX database	https://apps.ncl.ac.uk/edetox/	A database of *in vitro* and *in vivo* skin penetration data for many compounds, including information on skin type, area and vehicle
EURL ECVAM collection – JRC data catalogue (biokinetics databases)	https://data.jrc.ec.europa.eu/collection/id-0088 • EURL ECVAM Fish In Vitro Intrinsic Clearance Database • EURL ECVAM Fish In Vivo Biotransformation Database • EURL ECVAM in vitro hepatocyte clearance and blood plasma protein binding dataset for 77 chemicals • EURL ECVAM Rodent In Vitro Intrinsic Clearance Database • EURL ECVAM Rodent In Vivo Biotransformation Database	Databases with *in vitro* or *in vivo* information on fish and rodent clearance and biotransformation. And *in vitro* human data on hepatic clearance and fraction unbound for 77 chemicals.
Evolvus: Microsomal Stability Database	http://www.evolvus.com/products/databases/microsomalstability.html	Liver microsome stability assay data (Cl_{INT} and t½) for drugs and drug-like compounds curated from literature for rat, mouse, human and dog)

Goodman and Gilman's The Pharmacological Basis of Therapeutics 13th Edition	McGraw-Hill Publishers (2017) ISBN-13: 978-1259584732	Appendices provide key pharmacokinetic data for commonly used drugs e.g. oral bioavailability, urinary excretion, % bound in plasma, clearance, volume of distribution, half-life, Tmax and Cmax
Hazard Evaluation Support System and Integrated Platform (HESS)	http://www.nite.go.jp/en/chem/qsar/hess-e.html	Metabolic maps and ADME data for humans and rats
KinParDB European Commission Joint Research Centre	EURL ECVAM Kinetic Parameters Dataset	Kinetic parameters (e.g. clearance, half-life, AUC) for 100 diverse chemicals
Laboratory of Molecular Modeling and Design (LMMD) Datasets	http://lmmd.ecust.edu.cn/	ADME databases curated from the literature with information on blood brain barrier (BBB) partitioning, human intestinal absorption, P450 inhibitors and non-inhibitors
UFZ-LSER online database	http://www.ufz.de/lserd	Calculates partition coefficients from a CAS or SMILES
The Merck Index On-line	https://www.rsc.org/merck-index	Provides links to original publications for individual drugs, including detailed reports for pharmacokinetics
METRABASE[F]	http://www-metrabase.ch.cam.ac.uk/	Data on interactions between chemicals and proteins relating to metabolism and transport; 20 transporters and 13 CYP enzymes; identifies substrates and non-substrates / inhibitors and inducers
Obach et al., 2008	http://dmd.aspetjournals.org/content/36/7/1385	Clinical IV data
OECD QSAR toolbox	https://www.qsartoolbox.org/	Encompasses a collation of databases including data on plasma protein binding, absorption, rat and human metabolic data – skin and liver
On-line chemical modelling environment -oCHEM	https://ochem.eu/home/show.do	Datasets for many ADME properties (e.g. absorption, BBB partitioning, Caco2 permeability, log P, log D, water solubility, plasma protein binding, IC50, CYP Inhibition, PgP substrate activity; tissue:blood partition coefficients and time dependent tissue-drug concentrations

PharmaInformatic: PACT-F PPB-DB	http://www.pharmainformatic.com/html/pact-f.html	PACT-F provides bioavailability data for humans (from clinical trials) and preclinical animal studies. PPB-DB provides protein binding information
Pharmapendium: Elsevier	https://www.elsevier.com/solutions/pharmapendium-clinical-data	ADME information searchable by terms such as % absorption, bioavailability, cell / protein binding metabolic transformation, tissue distribution, volume of distribution, clearance, half-life; humans, birds, fish and mammals
pkCSM	http://biosig.unimelb.edu.au/pkcsm/	Caco-2 / skin permeability, HIA, P-gP / CYP substrate / inhibitor; clearance, renal OCT2 substrate; volume of distribution, BBB permeability, fraction unbound in plasma
QikProp	https://www.schrodinger.com/products	Predicts ADME relevant properties (e.g. blood brain partitioning, protein binding Caco-2 and MDCK permeability)
SwissADME	http://www.swissadme.ch/	Multiple ADME–related properties including GI absorption, BBB penetration, skin penetration, interactions with P-gP and CYPs, drug-likeness characteristics[P]
TRANSFORMER	http://bioinformatics.charite.de/transformer/index.php?site=home/	Information on metabolism and transport of compounds in humans
UCSF-FDA Transportal	http://transportal.compbio.ucsf.edu/about/	Information on transporter expression, location, substrates, inhibitors and interactions
US FDA drug database - drugs@fda (Orange Book)	https://www.accessdata.fda.gov/scripts/cder/ob/index.cfm/	*In vitro* and *in vivo* ADME data
VolSurf	http://www.moldiscovery.com/software/vsplus/	Creates 128 molecular descriptors from 3D Molecular Interaction Fields (MIFs) related to ADME
Sayre et al., 2020	https://www.nature.com/articles/s41597-020-0455-1.epdf?sharing_token=2o63aAL-k2ORopEOgsoVotRgN0jAjWel9jnR3ZoTv0PpoIaxrtXda2RFilzNF0yjalwHXFvSZiY14szzwt-f2UnnvvrE5iplCGAHReANtHA6eZxxKpTgTR6gN3TjkyCYMb04uCkhCzsnRIFemTWgSEC30JFXJFN-UCaed8vEn20%3D	Database of pharmacokinetic time-series data and parameters for 144 environmental chemicals

Table 1C. Dedicated PBK modelling software

Software	Available from	Brief summary of capabilities
Cloe	Cyprotex https://www.cyprotex.com/insilico/physiological_modelling/cloe-pk/	Predicts concentration-time profiles in plasma and 14 organs/tissues using *in vitro* ADME and physicochemical data; models available for human, rat and mouse
Cosmos KNIME workflow	http://www.cosmostox.eu/home/welcome/	Physiologically-Based Kinetic (PBK) models to simulate concentration-time profiles and internal dose metrics for dermal or oral exposure scenarios
High Throughput Toxicokinetics (Httk)	https://cran.r-project.org/web/packages/httk/index.html	Provides data tables and functions for simulation; facilities to parameterise PBK and one-compartment TK models for multiple chemicals and species; *in vitro* to *in vivo* extrapolation of HTS data; models can be exported for use with other simulation software
GastroPlus	Simulations Plus, Lancaster, CA https://www.simulations-plus.com/software/overview/	Comprises 10 modules including: PBPKPlus – enables PBPK modelling and IVIVE, can be parameterised for different disease states and age groups. ADMET Predictor – predicts physicochemical and ADME properties. Additional Dosage Routes – simulates oral cavity, dermal, pulmonary ocular and intramuscular administration. PKPlus – estimates PK parameters
Software	**Available from**	**Brief summary of capabilities**
Cloe	Cyprotex https://www.cyprotex.com/insilico/physiological_modelling/cloe-pk/	Predicts concentration-time profiles in plasma and 14 organs/tissues using *in vitro* ADME and physicochemical data; models available for human, rat and mouse
Cosmos KNIME workflow	http://www.cosmostox.eu/home/welcome/	Physiologically-Based Kinetic (PBK) models to simulate concentration-time profiles and internal dose metrics for dermal or oral exposure scenarios

High Throughput Toxicokinetics (Httk)	https://cran.r-project.org/web/packages/httk/index.html	Provides data tables and functions for simulation; facilities to parameterise PBK and one-compartment TK models for multiple chemicals and species; *in vitro* to *in vivo* extrapolation of HTS data; models can be exported for use with other simulation software
GastroPlus	Simulations Plus, Lancaster, CA https://www.simulations-plus.com/software/overview/	Comprises 10 modules including: PBPKPlus – enables PBPK modelling and IVIVE, can be parameterised for different disease states and age groups. ADMET Predictor – predicts physicochemical and ADME properties. Additional Dosage Routes – simulates oral cavity, dermal, pulmonary ocular and intramuscular administration. PKPlus – estimates PK parameters
IndusChemFate (CEFIC LRI)	http://cefic-lri.org/toolbox/induschemfate/ (Microsoft Excel spreadsheet files)	Generic PBK model (first tier or screening level tool); estimates tissue body fluid concentrations following oral, dermal or inhalational exposure to volatile or semi-volatile chemicals
MEGen	http://xnet.hsl.gov.uk/megen	Web application to generate PBK model equations; parameters may be retrieved from the integrated database or obtained from literature; output available in MATLAB, MCSim, R or other format
PBPK Model	https://tuspace.ca/~mparnis/files/PBPK200.html	The Canadian Centre for Environmental Modelling and Chemistry; Excel-based PBPK spreadsheet, parameterised for human male
Simcyp Simulator	Certara, Princeton New Jersey https://www.certara.com/software/physiologically-based-pharmacokinetic-modeling-and-simulation/simcyp-simulator/?ap%5B0%5D=PBPK	PBK modelling and simulation platform; links *in vitro* data to *in vivo* ADME to predict PK/PD interactions for small molecules and biologics. Incorporates databases of genetic, physiological and epidemiological information to enable simulation of different populations (includes modules for paediatrics and rat, dog and knock-out mouse). Predicts ADME parameters such as oral, dermal, pulmonary absorption, clearance. Includes: ADAM (advanced dissolution, absorption and metabolism) model – predicts variability in bioavailability using physicochemical properties and *in vitro* data; dissolution (from various dosage forms) for oral absorption; models also available for skin and pulmonary absorption; BBB partitioning, metabolism, clearance etc

PK-Sim and MoBi	Open Systems Pharmacology Suite (Bayer) http://www.systems-biology.com/products/PK-Sim.html	PK-Sim: PBK modelling tool with integrated database of anatomical and physiological parameters for humans, mouse, rat, dog and monkey. Uses interchangeable building blocks to enable alternative scenarios to be considered e.g. changing from animal model to human population or i.v. dose to controlled release. Mobi: Software for multiscale physiological modelling and simulation. A range of biological models can be imported (e.g. PBK model imported from PK-Sim) or developed de novo; Software is compatible with Matlab and R.
PLETHEM (Population Lifecourse Exposure-To-Health-Effects Model)	ScitoVation http://scitovation.com/plethem.html	Open source R package incorporating: a generic 11 compartment diffusion limited PBPK model; a high-throughput IVIVE model to extrapolate *in vitro* measured point of departure to equivalent exposures; an in-vitro to in-vivo model to extrapolate *in vitro* measured metabolism values to predicted *in vivo* values; population variability modelling; databases of age-dependent physiological and metabolic parameters; QSAR models to estimate partition coefficient
Monolix (Lixsoft)	http://lixoft.com/products/monolix/	publicly available for non-commercial purposes - Monolix is the most advanced and simple solution for non-linear mixed effects modeling (NLME) for pharmacometrics. It is based on the SAEM algorithm and provides robust, global convergence even for complex PK/PD models. Monolix is used for preclinical and clinical population PK/PD modeling and for Systems Pharmacology. Monolix is widely used by academia, the pharmaceutical industry as well as the US regulatory agencies.

Table 1D. Mathematical modelling and simulation tools that can assist PBK modelling

Resource	Available from	Brief summary of capabilities
A4S (Accelera for Sandwich)	Reported in publication of Germani et al (2013): https://doi.org/10.1016/j.cmpb.2012.10.006	Matlab based PK/PD simulator (incorporates 10 PK models; generates plasma concentration-time profiles, AUC, Cmax, t½ etc)
ADAPTS	Biomedical Simulations Resource, University of Southern California, https://bmsr.usc.edu/software/adapt/	Individual and population PK/PD modelling application
Berkley Madonna	Berkeley, CA https://berkeley-madonna.myshopify.com/	Generic differential equation solver capable of constructing complex models; automatic graphing of results; parameter estimation from curve fitting; sliders can investigate influence of changing different parameters
Biokmod	http://diarium.usal.es/guillermo/biokmod/	Mathematica-based packages for modelling linear and non-linear biokinetics; differential equation solver
chemPKTM V.2	Cyprotex, Cheshire, UK https://www.cyprotex.com/insilico/physiological_modelling/chempk	Predicts oral and i.v pharmacokinetic data from structure, using a KNIME workflow; calculates 10 tissue partition coefficients, absorption, renal clearance and metabolism; predicts clearance, t½ volume of distribution AUC, Cmax, Tmax etc
GastroPlus	Simulations Plus, Lancaster, CA https://www.simulations-plus.com/software/overview/	PKPlus module – estimates PK parameters for 1, 2 3-compartment or non-compartmental models; fitted parameters include 1st order absorption rate, lag time and bioavailability (can be linked back to GastroPlus model)
GNU MCSIM	GNU project https://www.gnu.org/software/mcsim/mcsim.html	Generic modelling and simulation program; solves user specified linear and nonlinear equations

INTELLIPHARM	Intellipharm, LLC, Niantic, USA https://www.intellipharm.com/physiologically-based-pharmacokinetic-modeling.htm	Combines simulation of drug dissolution, precipitation, absorption and gastric motility with bioavailability, clearance, and volume of distribution as coupled differential equations; provides open source code for PBK models.
Matlab (SimBiology)	MathWorks, Inc., Natick, MA https://www.mathworks.com/products/matlab.html	Modelling and simulation tools focussed on PK/PD and systems biology; library of common, customisable PK models; simulates time course of chemicals; model parameters estimated by fitting to experimental data; individual or population models; sensitivity analysis
Maxsim2	http://www.maxsim2.com/	Interactive PK/PD modelling software enabling investigation of consequences of varying physico-chemical, physiological or anatomical features; incorporates common PK and PD models.
NONMEM (including PREDPP)	ICON, Dublin https://www.iconplc.com/innovation/nonmem/	NONMEM – generic package for simulating / fitting data; PREDPP provides subroutines for predicting PK/PD data.
R (RStudio)	The R Project from the R foundation https://www.r-project.org/about.html RStudio – integrated development environment for R https://www.rstudio.com/products/rpackages/	Freely available software with a network of users continually adding new applications for use by the community; statistical analysis (linear and nonlinear); graphing techniques; for examples httk and PKfit for R
RVIS	Health and Safety Executive EPAA and CEFIC funded project http://cefic-lri.org/projects/aimt7-rvis-open-access-pbpk-modelling-platform/ https://github.com/GMPtk/RVis	RVis, a prototype application for the analysis of structure and performance of physiologically based pharmacokinetic (PBPK), and other models, written in the free, open source syntax MCSim or R.
Pheonix WinNonlin and Pheonix NLME	Certara, Princeton, New Jersey https://www.certara.com/wp-content/uploads/Resources/Brochures/BR_PhoenixWinNonlin.pdf	WinNonLin - Industry standard integrated tool for non-compartmental analysis, PK/PD modelling; NLME – non-linear mixed effect modelling and simulation software

PKfit for RF	https://cran.r-project.org/src/contrib/Archive/PKfit/	Pharmacokinetic tool for data analysis in R
PKPD Tools for Excel	Add on for Microsoft Excel http://pkpdtools.com/excel/downloads/	Add-on to assist PK/PD simulation and modelling within Microsoft Excel.
PopGen	Bayer http://xnet.hsl.gov.uk/popgen/	Virtual human population generator to predict realistic variation in anatomical and physiological parameters across populations.
Magnolia	https://www.magnoliasci.com/	
SAAM II (Simulation Analysis and Modelling) Version 2.3	TEG, The Epsilon Group, Virginia https://tegvirginia.com/software/saam-ii/	Development and statistical calibration of compartmental models; population kinetics; automatic generation of equations from model structure
SigmaPlot Transforms	http://www.sigmaplot.co.uk/products/sigmaplot/transforms.php	Resource for manipulating data within a worksheet; plotting, transforming and fitting data

Annex 2. Prospective use of microphysiological systems in PBK models

Organ on a Chip (OoC) models aim to recapitulate aspects of human physiology and pathology for use in drug discovery, efficacy and safety testing, and personalised medicine, with the goal to improve upon existing bioassays and provide insights into the mechanisms underlying the development and progression of diseases. In addition, OoCs are considered relevant to reduce the need, cost, and ethical burden of animal studies (Mastrangeli et al, 2019).

Although still in their infancy, it can be anticipated that OoC models, also known as microphysiological systems (MPS), will eventually provide an experimental basis for parameterising PBK models, especially in cases where *in vivo* data are lacking, and where there is a need to overcome drawbacks with current *in vitro* (static) systems. For example, disposition kinetics are mainly regulated by enzyme and biliary excretion and these parameters are experimentally estimated by using primary hepatocytes (Sivaraman et al., 2005) which do not recapitulate the full physiology of the liver organ compartment including enzyme activity and bile-duct. Similarly, Caco-2 cell culture model is used as a model of intestinal epithelial cells, but their villi-like structures and CYP3A4 activity is limited (Kim and Ingber, 2013). MDCK cells, which are commonly used for permeability studies, lack the glomerular or tubular structures of the kidney (Fagerholm, 2007). The status of OoC devices has been reviewed in the literature (Marx et al., 2016; Zhang and Radisic, 2017; Ishida, 2018; Kimura et al., 2018).

A critical feature for considering MPS as a means of recreating physiologically relevant organ (or tissue) compartments and biological functions is the use of microfluidics and mechanical stimulation (e.g., sheer stress, peristaltic motion) which differentiate OoC methods from conventional static cultures. In addition to traditional cell-lines, primary cells, spheroids, organoids, and Induced Pluripotent Stem Cell (iPSC)-derived tissue-like cells are used, depending on the context of application (Marx et al., 2016; Tetsuka et al., 2017). As summarised in Table 2A, several tissue MPS models have been published and are expected to provide more robust parameters for PBK model analysis.

Table 2A. Examples of microphysiological systems that could provide PBK model parameters

Tissue	Description	Reference
Gut	Primary human small Intestine-on-a-Chip using biopsy-derived organoids	Kasendra et al., 2018 Kim & Ingber, 2013
Liver	Metabolite identification and PK evaluation of hydrocortisone using liver MPS	Sarkar et al., 2015
	Comprehensive analysis of ADME-related mRNA expressions in primary human hepatocyte spheroids	Bell et al., 2016
Kidney	Proximal tubule function using human fresh RPTEC model	Weber et al., 2016
	Renal-specific transporters expressed in the human pluripotent stem cell–derived kidney model	Bajaj et al., 2018
Skin	Co-culture of human skin cells	Wufuer et al., 2016
Lung	Vascularized lung chip	Huh et al., 2013 Jain et al., 2018

Multiple-tissues	*In vitro* PK model of diclofenac and omeprazole using Gut-Liver MPS	Bricks et al. 2015 Tsamandouras et al., 2018
	Four-organ-chip model	Maschmeyer et al., 2015 Yu et al, 2015
	Four-, seven- and ten-MPS models	Edington et al., 2018

In order to use a MPS to determine a biochemical parameter as an input to a PBK model for a data poor chemical substance for which *in vivo* data do not exist or are limited, the computational PBK modelling proceeds with two steps: first, a computational *in vitro* PBK model for the MPS is constructed, and then it is extrapolated from *in vitro* to *in vivo* (Abaci and Shuler, 2015; Stokes et al., 2015; Prantil-Baun et al., 2018).

For example, a computational multi-MPS linked PBK model has been constructed, which incorporated MPS compartments of liver and gut with parameters for the experimental conditions (e.g., flow rate, volume and surface area of gut MPS barrier). Multiple biochemical parameters (i.e., CLint,liver, CLint,gut and permeability coefficient) were estimated by multi-line curve-fitting (Tsamandouras et al., 2018). Other computational *in vitro* PBK models for multi-MPS up to ten-MPS connected model have also been reported (Yu et al., 2015; Edington et al., 2018).

For *in vitro – in vivo* extrapolation (IVIVE), a scale-up process is needed (Prantil-Baun et al., 2018). Scaling factors (SFs), which are ratios of biochemical parameters between *in vivo* and *in vitro* systems, are useful for this purpose (Somayaji et al., 2016). Once SFs are estimated for a range of known chemicals with well-characterised *in vivo* and available clinical data, then the SFs are potentially applicable to data poor chemical substances.

The use of MPS to determine biochemical parameters for computational PBK models still faces a number of challenges, including: a) further confirmation of predictive performance of the PBK models and IVIVE scaling methods using a wide variety of chemicals for which *in vivo* exposure data exist; b) development of further PBK models for non-oral routes of chemical exposure (dermal, inhalation, etc.); c) integration of physiologically representative MPSs on a generalised platform; d) increase of throughput and robustness by standardisation and automation (Sung et al., 2014; Stokes et al., 2015; Edington et al., 2018; Prantil-Baun et al., 2018). Generally speaking, a quality assessment framework will need to be developed.

Simulating chemical effects in humans remains the big challenge, although it can be anticipated that human iPSC derived single organ MPS will be integrated with PBK models in the near future. Another challenge is to assess inter-subject variability in parameters by using individual MPSs ("you-on-a-chip"). Finally, as a long-term perspective a generalised multi-MPS platform (whole-body, human-on-a-chip) may be used as a wet PBK simulator, as a replacement for *in vivo* studies.

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Annex 3. Sensitivity analysis details

Uncertainty analysis - Latin Hypercube (LH) sampling

Latin Hypercube (LH) sampling is a statistical distribution sampling tool that can be used to perform an efficient analysis of model structure uncertainty (Olsson et al., 2003). In simple terms, each parameter distribution is divided into *m* regions of equal probability with a point sampled from each region and the *m* points for each parameter randomly matched to produce *m design points*. Criteria such as maxi-min (Morris & Mitchell 1995) and orthogonality (Tang, 1993) can be applied to create Latin Hypercube Designs that better explore parameter space for a given sample size *m*.

LH sampling can be applied to study whether PBK model behaviour in a defined parameter space is broadly reasonable, i.e. is the broad shape of concentration-response curves for various model outputs physiologically plausible for the LH sample? This has the effect of "stress" testing model behaviour by efficiently sampling from the edges of multidimensional parameter space. The variability in model evaluations observed over an LH sample may identify features of the model (such as magnitude of peak concentration, time to reach peak concentration, time spent above a given threshold concentration) that might need to be quantitatively studied through sensitivity analysis. Furthermore, by rejecting a subset of samples that are physiologically implausible, the parameter distributions can be revised (McNally et al, 2018). An evaluation of absorption, distribution and metabolism can be undertaken by studying three output measures, such as venous blood concentrations of parent chemical and metabolite, and urinary excretion of metabolite.

Concentration-response profiles corresponding to a LH sample are shown for the concentration of parent chemical and metabolite in venous blood (mg/L) and the concentration of metabolite expressed relative to creatinine in urine (mg/g creatinine) in Figures 3A to 3C respectively. These simulations indicated a maximum concentration of both parent chemical and metabolite in venous blood were rapidly achieved following ingestion and fell rapidly following a sharp peak. Apart from the magnitude of the peak, there was little qualitative difference in the concentration response profile over the runs (Figures 3A and 3C). Simulations of the metabolite in urine (Figure 3C) indicate larger differences in the timing and magnitude of peak concentration of metabolite in urine and in the rate of the subsequent decline in concentration. Overall, the simulations indicate the qualitative behaviour of the PBK model was reasonable over the ranges of the model inputs, which serves as a check on the coding of the model and the assumed distributions and ranges for parameters.

Figure 3A. Assessing the qualitative behaviour of a model: concentration time curve for a parent chemical in venous blood

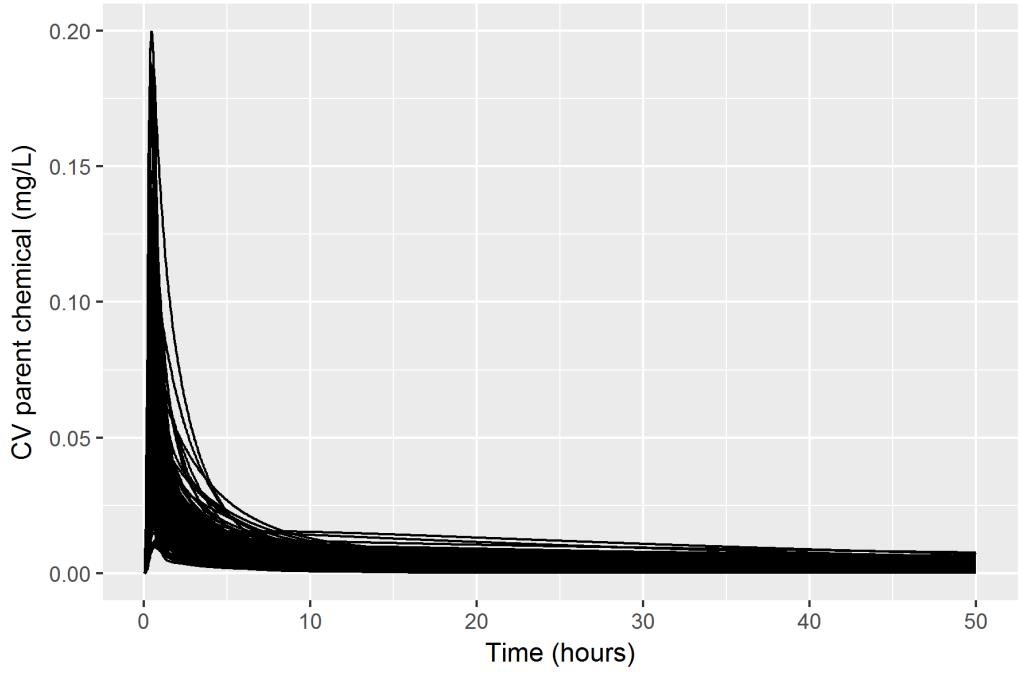

Figure 3B. Assessing the qualitative behaviour of a model: concentration time curve for a metabolite in venous blood

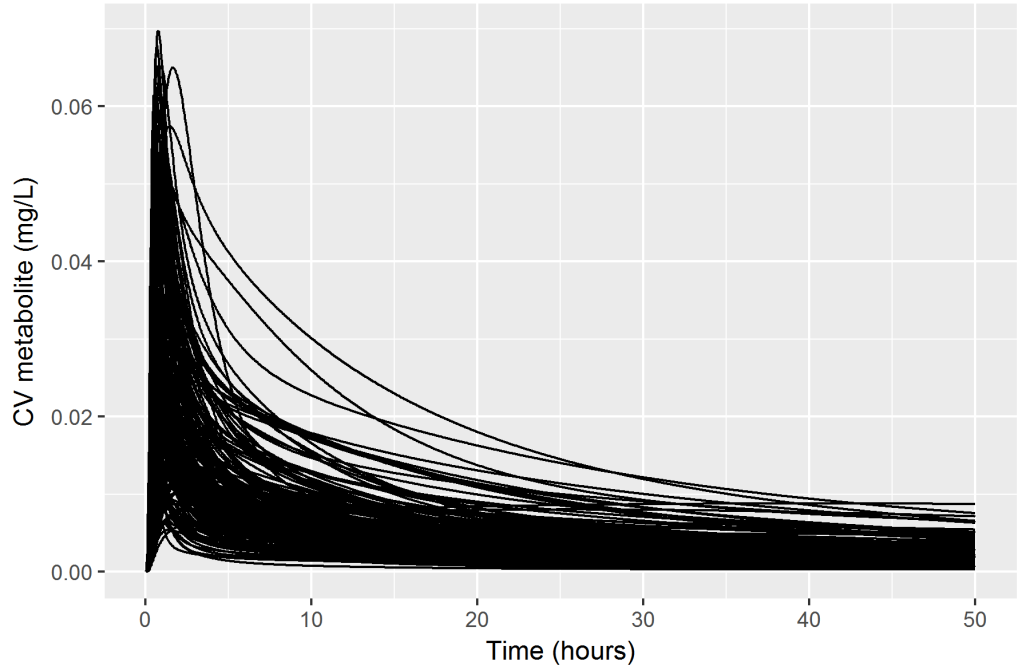

Figure 3C. Assessing the qualitative behaviour of a model: concentration time curve for a metabolite in urine

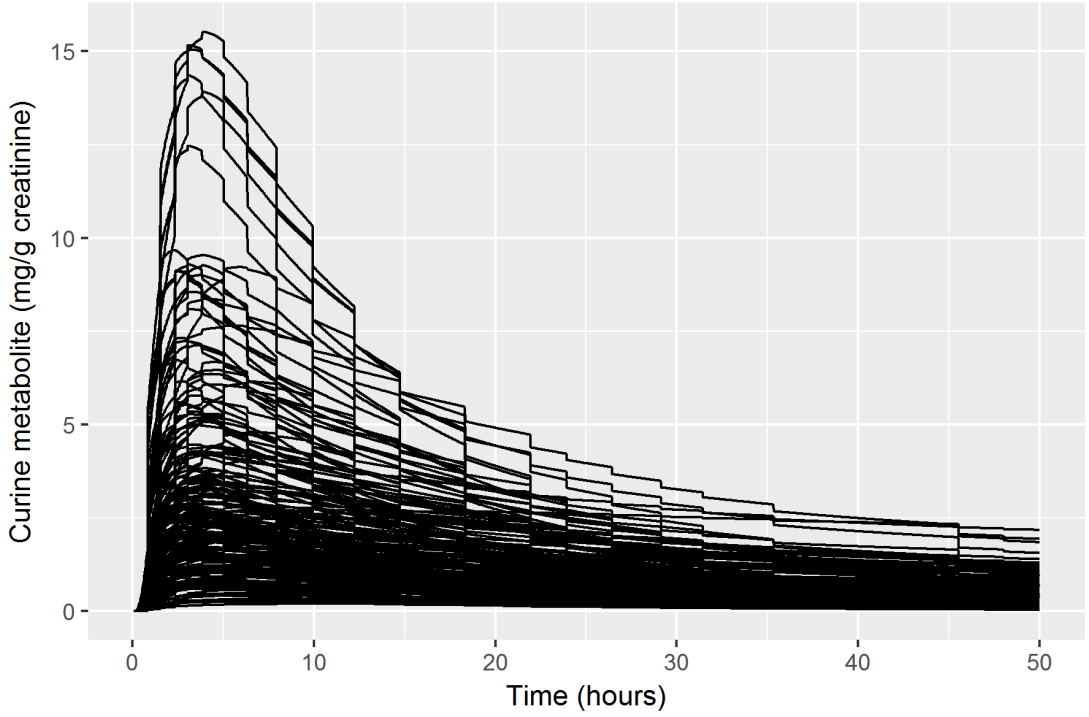

Table 3A. Presentation of PBK Model parameters for human sensitivity and uncertainty analysis

Human Physiological Parameters	Abbreviation	Mean	Distribution
Body weight (kg)	BW	72.3	N(72.3,9.05)
% BW			
Total vascularised tissues	VT	0.95	-
Liver	VLiC	3.09	N(0.0311, 0.008)
Fat	VFaC	19.5	ln(-1.59,(-2.88)^2)
Gut	VGuC	1.50	U(1.19,1.84)
Stomach	VStC	0.22	N(0.002, 0.0007)
Slowly perfused tissue	VSpdC	60.7	N(60.7, 9.4)
Rapidly perfused tissue	VRpdC	3.71	N(3.7, 0.26)
Blood	VBldC	5.0	U(2.5,10)
Cardiac output (L h^{-1} kg^{-1} BW)	QCC	14	N(13.8, 2.5)
% Cardiac output			
Liver	QHepartC	6.0	N(6.89, 0.52)
Fat	QFaC	5.0	N(5.3, 0.3)
Gut	QGuC	14.9	U(13.2,16.6)
Stomach	QStC	1.1	N(1.1, 0.08)
Slowly perfused tissue	QSpdC	27.0	N(28.7, 1.91)
Rapidly perfused tissue	QRpdC	42.0	N(43.1, 2.78)

Metabolic Clearance (minutes)			
In vitro half-life MINCH	$T_{½minch}$	30.53	N(30.54, 2.39)
Microsomal protein yield (mg g^{-1})			
Hepatic	MPY	34	N(34,15)
Gut	MPY_{gut}	3.9	U(1.95, 7.8)
Fraction Bound in plasma (proportion)			
Parent chemical	FBparent	0.000125	U(10^{-5}, 0.01)
Metabolite	FBmetabolite	0.014648	U(0.001, 0.01)
Gastric emptying (h^{-1})			
Maximum	$k_{(max)}$	10.2	U(5.1, 20.4)
Minimum	$k_{(min)}$	0.005	U(0.0025, 0.01)
Absorption (h^{-1})[4]			
Gut	k_{Ga}	25.1	U(12.55, 50.2)
Urinary production (L h^{-1})	Rurine	0.1	N(0.104, 0.053)
Creatinine concentration (g L^{-1})	Creat	0.5	N(1.278, 0.605)
Urinary excretion rate (h^{-1})	K1	0.15	U(0.05, 2)

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Annex 4. List of Case studies developed in 2020 to accompany this Guidance

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Case study	PBK model	Application
I	Generic PBK model for farm animal species: Cattle (*Bos taurus*), Swine (*Sus scrofa*), Sheep (*Ovis aries*) and Chicken (*Gallus gallus domesticus*)	Chemical risk assessment in food and feed safety aims to set safe levels of regulated compounds and contaminants to protect farm animals after exposure through feed, and to protect humans against carry over and residues in animal products (e.g. meat, milk, eggs).
II	Generic PBK model for four fish species: rainbow trout, zebrafish, fathead minnow, and three-spined stickleback	Environmental risk assessment of chemicals for the protection of fish species
III	PBK model for rats	In vitro-to In vivo extrapolation (IVIVE) for potential endocrine disrupting compounds
IV	PBK model for methyleugenol and estragole in humans	Forward dosimetry. Human health risk assessment of alkenylbenzenes occurring naturally in certain herbs. Illustrates the use of the read-across approach in model validation.
V	PBK model for acrylonitrile in humans	Interspecies extrapolation (rat to human). Forward dosimetry. Human health risk assessment.
VI	PBK model for monoisononyl phthalate in humans	Interspecies extrapolation (mouse to human). Forward dosimetry. Human health risk assessment. Setting of blood-based biomonitoring equivalents for phthalates
VII	PBK model for statins (HMG-CoA reductase inhibitors) in humans	Forward dosimetry. In vitro-to In vivo extrapolation (IVIVE) of transporter kinetics based on proteomics
VIII	PBK models for caffeine in rats and humans	Interspecies and route-to-route extrapolation. Illustrates concept of internal margin of exposure
IX	PBK models for caffeine in rats and humans	Interspecies and route-to-route extrapolation applying read across information
X	PBK models for seven structurally related phenyl-1,4-dihydropyridine compounds (calcium channel antagonists) in humans	Forward dosimetry. Human health risk assessment. Illustrates the use of the read-across approach in model validation.
XI	PBK model for herbicides in rats	Forward dosimetry. Oral bioavailability estimation.
XII	PBK model for three chemicals (coumarin, caffeine and sulforaphane) in humans	Forward dosimetry. Human health risk assessment of dermally applied products.
XIII	Generic one compartment model and PBK model for humans	Forward dosimetry. Human health risk assessment of chemicals (contaminants) in food.

The fields of toxicology and chemical risk assessment evaluate the safety of chemicals for humans the environment. Increasingly, modern methods seek to reduce the use of animals in chemical safety testing and predictive toxicology.

In this context, the OECD has developed this guidance document on Physiologically Based Kinetic (PBK) models, with the goal of increasing confidence in the use of these models parameterised with data derived from *in vitro* and *in silico* methods.

The document provides insights into how the data generated by such methods can be applied to construct PBK models and how these models can be validated. A series of cases studies illustrate the use of PBK models based on *in vitro* and *in silico* data, along with the application of the model assessment framework proposed herein.

This guidance document provides a clear and consistent model assessment framework for facilitating the dialogue between the developers and proponents of PBK models and regulators who review and adopt the use of PBK models.

oe.cd/risk-assessment