HBM4EU science and policy for a healthy future

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Roadmap for PBTK/TD model refinement and analysis for priority substances

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WP 12 - From HBM to exposure
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2 Work Package 12: From HBM to exposure

The main objective of this WP is to link human biomonitoring (HBM) data to external exposure. The work will link data from human biomonitoring, environmental monitoring and external exposure modelling. This will support a more effective interpretation of HBM data in elucidating chemical exposure and supporting both chemical risk assessment and management as well as advanced research in the association between environmental burden and public health.

The work will help to determine the external exposure levels for the HBM4EU priority substances, starting from HBM data and using a reverse dosimetry approach. This will contribute to the identification of external exposure levels in Europe that are above health-relevant values, facilitating thus decision-making regarding risk control measures. When coupled with regulatory multi-media environmental models this approach would also support the setting of safety levels in different environmental media. Available human physiology-based toxicokinetic (PBTK) models will be reviewed and analyzed to properly parameterize a generic PBTK modelling platform for the priority substances, both individually and in combination. Both the biochemical interactions between components of chemical mixtures to which the EU population may be exposed, as well as changes in absorption, distribution, metabolism and excretion (ADME) properties and internal exposure processes with age and gender will be taken into account.

This new knowledge will allow the HBM4EU team to assess newly proposed regulatory thresholds and to determine which exposure pathway(s) and route(s) contribute the most to the overall exposure burden.

Existing exposure-related and ancillary data for HBM4EU priority substances and state of the art exposure models will be collated and adapted to support the estimation of regional differences in exposure. Exposure models will be coupled to PBTK modeling to effectively translate the estimated exposure levels into internal and biologically effective dose at target tissues and candidate biomonitoring matrices. Thus, the biologically effective dose of xenobiotics that is related to the onset of adverse outcome pathways can be linked to both biologically monitored levels and to external exposure levels. This would be expected to increase the relevance and applicability of the AOP framework of the OECD for the priority compounds targeted in HBM4EU.

3 Task 12.3: Refinements of toxicokinetic modelling

PBTK models are quantitative descriptions of the ADME of chemicals in biota based on the interrelationships among key physiological, biochemical, metabolic and physicochemical determinants of these processes.

The process of PBPK model development can be described in the following interconnecting steps[1]:

1) **Problem formulation** and data evaluation
2) **Model structure and characterization** which involves the development of conceptual and mathematical descriptions of the relevant compartments of the human or animal body as well as the exposure and metabolic pathways related to the chemical under study;

[1] IPCS harmonization project document no. 9 (2010): Characterization and application of physiologically based pharmacokinetic models in risk assessment. See: http://www.who.int/ipcs/methods/harmonization/areas/pbpk_models.pdf?ua=1

3) **Model parameterization** which involves obtaining quantitative estimates of measures of the mechanistic determinants (e.g. anatomical, physiological, physicochemical, biochemical parameters);
4) **Mathematical and computational implementation**
5) **Model simulation, i.e. simulation of the kinetics**;
6) **Model refinement** and if necessary loop back to steps 3, 4 and 5
7) **Model evaluation & validation** which involves comparison of the a priori predictions of the PBPK model with experimental data as well as conducting uncertainty, sensitivity and variability analyses to refute, support or refine the model description and parameters.

Appropriate validation and/or refinement will allow a successful use of PBTK models to estimate internal and biologically effective dose in human target tissues and/or HBM-related matrices, but also to conduct extrapolations of the toxicokinetic (TK) behaviour of chemicals from one route of exposure to another, from high dose to low dose and from one species to another. Model refinements can be performed according to parameters such as age, exposure routes, physicochemical properties and type of tissue.

Our suggestions on the process for determining whether a model needs to be refined or not will be detailed here below, according to key principles and best practices in PBTK modelling, which are essential for the characterization and application of PBTK models in health risk assessment.

A next step of the task 12.3 will be to perform refinements of PBTK/TD models currently available for the HBM4EU priority compounds, if it appears necessary from the steps described here below.

4 Roadmap for PBTK/TD model analysis & refinement

The aim of the roadmap presented hereby is to describe if and how a model has to be refined. This roadmap respects the key principles and best practices for characterizing and applying physiology-based pharmacokinetic (PBPK) models in risk assessment, described by the World Health Organization (WHO) on Characterization and Application of Physiologically based Pharmacokinetic Models in Risk Assessment (2010), a project conducted within the International Programme on Chemical Safety (IPCS). However, it extends the IPCS framework as the scope of using PBTK/D models in HBM4EU goes beyond performing chemical risk assessment for regulatory purposes.

The roadmap starts by listing the general information and characteristics of PK/PBPK or PD/PBPD models that should be considered to assess the reliability of the model. These characteristics include toxicokinetic and ADME parameters (e.g. tissue-blood partition coefficients, metabolic constants, clearance rates) or key toxicodynamic events (e.g. enzyme induction, binding protein induction, cofactor depletion). In a second step, evaluation of the parameters must have been performed by the authors in terms of sensitivity and uncertainty analyses. In the opposite case, this has to be highlighted as information gap. This process will inform on the level of confidence of the model and lead to indications on the model refinement needs.

4.1 General information on the model: purpose and model description

4.1.1 Problem formulation and data evaluation

Many human PBTK and to a lesser extent PBTK/TD models have been set up originally for risk assessment purposes often by re-parameterising an animal PBTK model that was based on animal data (WHO IPCS, 2010). In HBM4EU the intended uses of PBTK/D modelling are as follows:

(a) One potential use is to try to link directly to external exposure models to improve prediction of blood/plasma and urinary excretion levels in order to compare those predictions to HBM measured data (in cases where internal exposure is not given as a measured value but as a value predicted from the external exposure model). This would allow for extrapolations of

HBM-based guideline values to wider population pools supporting the EU-wide use of HBM data for policy making.

(b) Another one is to assist in the quantification of internal and biologically effective dose both on a systemic level and at target tissues that can be linked to biological markers of preclinical effects that will be measured in HBM4EU (in WP14 - "HBM effect biomarkers"). That can be related to AOP development and the quantification of effect biomarkers in conjunction with WP13 and thus enhance our capability to related exposures to adverse health outcomes.

(c) A third purpose is to perform external exposure reconstruction by performing reverse dosimetry modelling based on measured HBM data. In this way, HBM data can be used for external exposure quantification and thus provide the basis for exposure and risk management measures on the policy level.

The different intended uses of PBTK models in HBM4EU might have consequences on the identification of criticalities in the original PBTK and/or PBTK/D model. They have to be addressed and clearly distinguished from each other in the problem formulation phase and taken into account in relation to availability of data for evaluation and validation purposes. Aspects inferred from this problem formulation and data evaluation phase will have consequences for the following steps in using, amending, implementing, running, refining, evaluating the existing PBTK and/or PBTK/D models for refinement and analysis of priority substances. This roadmap refers primarily to refinement of the model parameter values and the respective parameterization scheme. Model structure evaluation and eventual need for re-structuring will be tackled mainly in the model review undertaken in task 12.1 of HBM4EU.

4.1.2 Scope and purpose of the model

The scope for the use of a PBPK model in a particular risk assessment essentially determines the intended model capability and the extent of model evaluation. Therefore, it is critical to clearly identify the type of risk assessment it is intended to support, the aspects of the assessment it is designed to facilitate, as well as the mode of action (MOA) hypotheses and associated weight of evidence underlying the model structure (e.g. toxicity from a reactive metabolite versus receptor binding).

The structure of a PBPK model, the level of details and parameterization depends in large part upon the purpose for which the model is developed and the available data.

The purpose and capability of PBPK models should be thus characterized in terms of the life stage, exposure routes/window and dose metrics that are central to their application in risk assessment (IPCS 2010).

4.1.3 PBTK model description

Table 1 - PBTK model description

PBTK model description				
Type of information	**Should contain**	**Answer (to be filled in)**	**Comments**	**Suggestion for model improvement**
Substance name	(Name, CAS number)			
Authors + years of publication				
Purpose of the model				
Model Code				

Target population	Human (adult, life stage, gestational)		It is suggested first to assess the refinements need on human PBPK models for priority compound	
Route of exposure	(Inhalation, oral, dermal)			
Dose metric selected and coherence with problem formulation	($AUC_{0\text{-}24h}$, steady-state concentration in blood or concentration in urine preferably expressed relative to creatinine excretion or urine density)			
Number, description and type of compartments	If possible, description of uptake compartments If possible, indications on whether compartments are well stirred or whether the uptake by an organ is permeability rate limited (should be consistent for highly bound compounds where plasma and interstitial space must be separately defined within the model)			
Metabolic scheme	Number of metabolites Description of the metabolic scheme showing the different pathways and metabolites Accordance with known biochemical processes of the substance			
Physiological parameter **Type of parameter (e.g. tissue volumes, body weight, glomerular filtration rate, …)** **Method for parameterization**	Specification on the data or method used for parameterization (e.g. QSAR, in vivo data, in vitro data, curve fitting) and associated indicative level of confidence (see tables 1A & 1B on indicative level of confidence below) Specification whether the parameters are constant or if age- or/and sex dependent changes are considered		If constant, search equation that describes age-dependent changes in physiological parameters	
Physicochemical parameter				

Partition coefficient				
Biochemical parameter **Type of parameter (e.g. metabolic rates as Vmax, Km, GEC, MET, EHR, …)** **Method for parameterization**	Specification on the data or method used for parameterization (e.g. QSAR, in vivo data, in vitro data, curve fitting) and associated indicative level of confidence (see tables 1A & 1B on indicative level of confidence below)			
Model calibration	Specification on the dose metric used for the model calibration References of the studies used for calibration			
Additional information	(i.e. Presence of enterohepatic recirculation)			
Biological plausibility of the model				
Remarks				

Indicative level of confidence for model parameter values

Please, note that the level of confidence attributed to the mode parameter values, according to the method used for their determination, could change depending upon the problem formulation.

Table 1A - For reverse dosimetry and forward dosimetry purpose

Indicative level of confidence for model parameter values	
High	Data **measured** from in vivo/in vitro studies (animal, human tissues)
Medium	Data **estimated** by optimisation/curve fitting
Low	Data **estimated** by other in silico method (QSAR,…)

Table 1B - For supporting AOP development and further use in linking exposure to health outcomes

Indicative level of confidence for model parameter values	
High	Data **measured** from human tissues
Medium	Data **measured** from in vivo/in vitro animal studies
Low	Data **estimated** by optimisation/curve fitting Data **estimated** by other in silico method (QSARs,k...)

4.1.4 Physiology-based toxicodynamic (PBTD) models description

Table 2 - PBTD model description

PBPD model description			
Type of information	**(Should contain)**	**To be filled in**	**To be expected a minima / further action needed**
Substance name	(CAS number)		
Authors + years of publication			
Mode of action (MOA) fully understood			
Toxicodynamic events is appropriate according to MOA			
Type of toxicodynamic events	Enzyme induction, binding protein induction, cofactor depletion….		
Effect metric selected is appropriate for the selected toxicodynamic events			
Toxicodynamic events parameterization / calibration	in silico, in vitro, in vivo		

4.2 Parameter verification and model analysis

The PBTK model should be capable of predicting the observed basic pharmacokinetics of the chemical (parent compounds or metabolites) before the model can be used for simulations of specific scenarios. Moreover, the acceptable prediction of dose metric should follow the acceptance criteria as indicated from the WHO guidance (IPCS, 2010) i.e. the ratio between simulated and observed data should be within a factor of 2. If the ratio between simulated and observed data (parent compounds and/or metabolites) is not within a factor of 2, it will then be necessary to refine and update the model with further toxicokinetic (ADME) data.

If a metabolic scheme is available, evaluation on how well the model describes the respective metabolic/biochemical processes (number of metabolites, metabolites tree) should be performed.

Sensitivity analysis is an important component of model verification, especially for uncertain parameters with a high potential to influence the outcome of the simulation. A sensitivity analysis must have had been performed by the authors for all parameters. If the sensitivity analysis was not performed by the authors, the model assessor will have to perform it (see section 4.3.1).

Uncertainty analysis, which evaluates the impact of the lack of precise knowledge of parameter values and model structure on dose metric simulations (IPCS 2010) must have had been performed by the authors. For parsimony, uncertainty analysis could be limited to the parameters identified through the sensitivity analysis as the ones that have the highest likelihood to affect the result of the

model calculations. If the uncertainty analysis was not performed by the authors, the model assessor will have to perform it (see section 4.3.2).

Table 3 - Parameter verification and analysis

Parameter verification and analysis			
Type of information	**Should contain**	**Answer (to be filled in)**	**Suggestion for model improvement**
Model verification			
Required information	(AUC in blood, urinary excretion rates or normalized urinary content) Prediction of the selected dose metrics and ratio of dose metric prediction towards observed parameters NB: according to the IPCS guidance, the dose metric prediction must be within 2 fold of observed parameters	Acceptable prediction of dose metric	Reference of the publication used for model verification If not, search data for this purpose & perform uncertainty analysis
Additional information	Description of the rational exposure scenarios (info from Risk Assessment Report might be required) Comparison of the model estimates with biomonitoring data (from literature at this stage) Simulation of potential dose dependence (e.g. testing non-linearity)		If a parameter value has been estimated, the data source and estimation method should be described
Model analysis			
Sensitivity analysis performed for all parameters	Time history / final value		If not, must be performed
Uncertainty analysis performed for the most influential parameters	Time history / final value		If not, must be performed

4.3 Model evaluation

4.3.1 Sensitivity analysis result

Sensitivity analysis provides a quantitative evaluation of how input parameters influence the dose metrics or other model output of relevance to the risk assessment, or to the problem as defined at the beginning (IPCS 2010).

Note that:

- time-dependent sensitivity analysis should be performed with the appropriate dose metric for compounds with half-lives shorter than 24h,
- final sensitivity analysis should be performed with the appropriate dose metric for compounds with half-lives longer than 24h.

Sensitivity analysis results (IPCS 2010) are summarized as:

- high (absolute value of normalized coefficient greater than or equal to 0.5)
- medium (absolute value of normalized coefficient greater than or equal to 0.2 but less than 0.5)
- low (absolute value of normalized coefficient greater than or equal to 0.1 but less than 0.2)

According to the results of sensitivity analyses, additional information will be needed for parameters with normalized sensitivity coefficients > 50% and refinement on the parameter with literature search (in vivo, in vitro data, QSAR) and/or the generation of new experimental data will have to be performed.

Table 4 - Sensitivity analysis

Physiological parameters		
Parameter name	**Parameter value**	**Sensitivity analysis result**
Blood flow		
Ventilation rate		
Body weight		
Tissues volume		
……		
Physicochemical parameters		
Parameter name	**Parameter value**	**Sensitivity analysis result**
Tissue:blood partition coefficients		
…		
Metabolic parameters		
Parameter name	**Parameter value**	**Sensitivity analysis result**
Michaelis-Menten maximal velocity (Vmax)		
Michaelis-Menten (Km)		
..		

Biochemical parameters		
Parameter name	**Parameter value**	**Sensitivity analysis result**
Renal clearance		
Protein binding		
…		

4.3.2 Uncertainty analysis

The notion of uncertainty encompasses both true uncertainty (i.e. in model parameter value) and variability (i.e. from population variability). Variability refers to inherent heterogeneity that is distributed within a defined population, such as body weight. In contrast, true uncertainty refers to a parameter that has a single value, which cannot be known with precision due to measurement or estimation error, such as partition coefficient.

The level of uncertainty is determined based on the ratio of the 95th percentile (P95) over the median value (P50) for the selected dose metric i.e., AUC, C_{max}, etc.

Uncertainty analysis results (IPCS 2010) are summarized as:

- high uncertainty (value could be a factor of 2 or higher)
- medium uncertainty (value could be a factor between 0.3 and 2)
- low uncertainty (value could be a factor of 0.3 or lower)

All parameters are potential candidates for refinement. However, only those with high uncertainty should be modified, however only within a reasonable range of biological plausibility.

Table 5 - Uncertainty analysis for the parameters

Physiological parameters of the model			
Parameter name	**Parameter value**	**Sensitivity analysis result**	**Uncertainty analysis result**
Blood flow			
…			
Physicochemical parameters			
Parameter name	**Parameter value**	**Sensitivity analysis result**	**Uncertainty analysis result**
Tissue:blood partition coefficients			
…			
Metabolic parameters			
Parameter name	**Parameter value**	**Sensitivity analysis result**	**Uncertainty analysis result**
Michaelis-Menten maximal velocity (Vmax)			
Michaelis-Menten (Km)			
..			
Biochemical parameters			
Parameter name	**Parameter value**	**Sensitivity analysis result**	**Uncertainty analysis result**

Renal clearance			
Protein binding			
…			

4.3.3 Coupling the results of sensitivity and uncertainty analysis

The outcome of sensitivity and uncertainty analyses might inform the reliability of a model to provide dose metric predictions of use in risk assessment, as illustrated in Figure 1 (IPCS 2010).

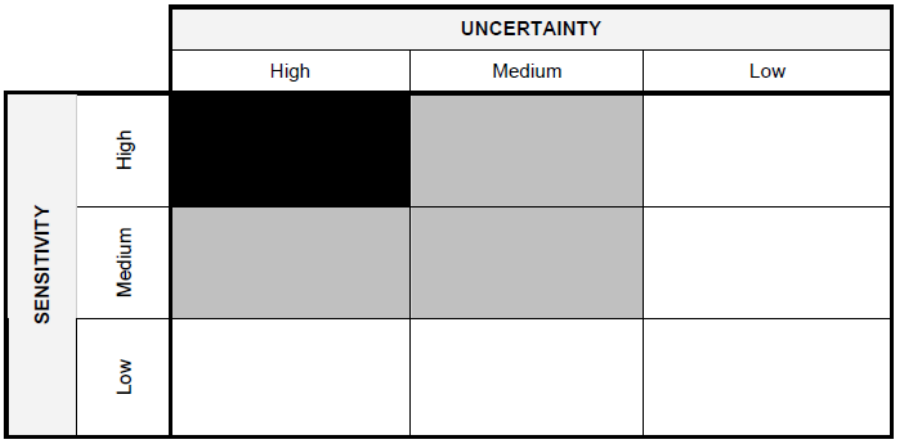


Figure 1- Illustration of the role of sensitivity and uncertainty analyses in determining the reliability of PBPK model predictions of dose metrics for risk assessment. Low reliability (black box); Medium reliability (grey boxes); high reliability (white boxes) (see IPCS 2010)

The reliability of the model predictions regarding dose metrics that can be used for risk assessment, where feasible, is based on the level of sensitivity of the predictions to the model parameters and the level of uncertainty of the parameter values. If the highly sensitive parameters are also the ones that are highly uncertain, then the reliability of the model for risk assessment applications would be questionable (IPCS 2010).

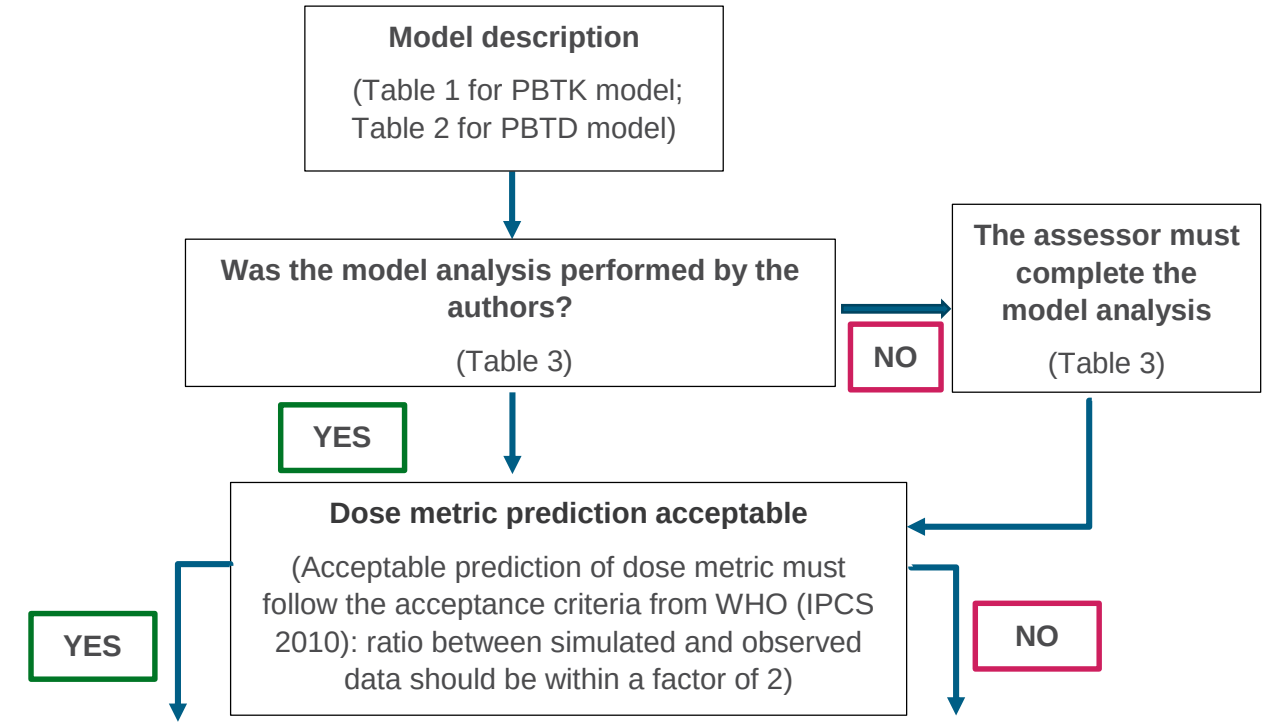
4.4 Model refinement and prioritisation

The level of confidence towards parameter values (see Tables 1A and 1B) together with the results of the sensitivity and uncertainty analysis for the parameters (see Table 4, figure 1) can be informative for assessing and prioritising the model refinement needs, as suggested from Table 6 here below. Indeed, additional information will be needed as a priority for a parameter with normalized sensitivity coefficient above 50% and high uncertainty and whose level of confidence towards its determination method is low (grey field of Table 6). Refinement on the parameter with literature search (in vivo, in vitro data, QSAR) and/or the generation of new experimental data will have to be most certainly performed.

Table 6 – Coupled Uncertainty and Sensitivity analysis for the parameters

		Uncertainty and Sensitivity analysis
		Normalized sensitivity coefficients > 50% and high uncertainty (value could be a factor of 2 or higher)
Estimated level of confidence of chemical specific parameter value	High	
	Medium	
	Low	

4.5 Flowchart - Roadmap for PBTK/TD model refinements need



No need for refinement

The model was checked by the authors and prediction seems acceptable.

Uncertainty and sensitivity analysis have been performed with the appropriate dose metric.

Need for refinement

Sensitivity analysis with the appropriate dose metric (Table 4) and uncertainty analysis (Table 5), if not already performed by the authors, should be done by the assessor.

The results of the coupled sensitivity and uncertainty analysis, crossed with the estimated level of confidence attributed to the chemical specific values can be informative for assessing and prioritising the needs for the model refinement (see Table 6), as refinements (with either literature search (in vivo, in vitro data, QSAR) and/or generation of new experimental data) will be needed as a priority for parameters with normalized sensitivity coefficient above 50%, high uncertainty and whose level of confidence towards their determination method is low.

Annex 1 - Roadmap for model refinement needs applied for a BPA model

Publication from:

Yang et al. 2015, Development of a physiologically based pharmacokinetic model for assessment of human exposure to bisphenol A, Toxicol. Appl. Pharmacol., 289 (2015), pp. 442-456

Available from:
http://www.sciencedirect.com/science/article/pii/S0041008X15301198/pdfft?md5=ea79c5cc6064feb5d989241dbb40f273&pid=1-s2.0-S0041008X15301198-main.pdf

1/ BPA model description

PBTK model description				
Type of information	**Should contain**	**Answer (to be filled in)**	**Comments**	**Suggestion for model improvement**
Substance name	(Name, CAS number)	Bisphenol A (BPA) 80-05-7		
Authors + years of publication		Yang et al., 2015		
Purpose of the model		Estimation of the inter-individual variability of internal dose metrics of BPA for the general population, based on the estimated daily intake of BPA in the United States		
Model Code		ACSLX (version 3.0.2.1) Code provided in the supplementary data section		Translation to R
Target population	Human (adult, life stage, gestational)	Adult		
Route of exposure	(Inhalation, oral, dermal)	Oral and dermal exposure	Dermal route not considered	
Dose metric selected and coherence with problem formulation	($AUC_{0\text{-}24h}$, steady-state concentration in blood, concentration in urine preferably expressed relative to creatinine	Concentrations of parent compounds (BPA) or metabolites (BPAG) in urine and blood		

	excretion or urine density)	Coherent with problem formulation		
Number, description and type of compartments	If possible, description of uptake compartments If possible, indications on whether compartments are well stirred or permeability rate limited (should be consistent for highly bound compounds where plasma and interstitial space must be separately defined within the model)	* 8 compartments for BPA: serum, liver, fat, gonads, richly perfused tissues, slowly perfused tissues, brain and skin * 2 sub-compartments (non-physiological) for BPAG and BPAS: volume of distribution, Vbody Well stirred compartment	Small intestine, stomach and gut are not to be considered as compartments (no indication on volume, or partition coefficient)	
Metabolic scheme	Number of metabolites Description of the metabolic scheme showing the different pathways and metabolites Accordance with known biochemical processes of the substance	2 metabolites : BPAG and BPAS		
Physiological parameter **Type of parameter (e.g. tissue volumes, body weight, glomerular filtration, …)** **Method for parameterization**	Specification whether the parameters are constant or if age-dependent changes are considered	See Table 1: from published literature or set to the study-specific values (for BW) or estimated (BMI) Constant parameters, except age-dependent Vfat		Possible refinement by using an equation describing the BW as an age-dependent change
Physicochemical parameter **Partition coefficient**		See Table 2		
Biochemical parameter **Type of parameter (e.g. metabolic rates as Vmax, Km, GEC, MET, EHR, …)** **Method for parameterization**	Specification on the data or method used for parameterization (e.g. QSAR, in vivo data, in vitro data, curve fitting) and associated indicative level of confidence	See Table 3 Human, in vitro / in vivo data		

Model calibration	Specification on the dose metric used for the model calibration References of the studies used for calibration	Serum and urine concentration for BPA, BPAG and BPAS * Thayer et al (2015): N = 11 subjects In a second step (revised re-calibrated mode): * Teeguarden et al (2015): N = 10 subjects		
Additional information	(Presence of enterohepatic recirculation)	Presence of enterohepatic recirculation	Biological basis of model development is questionable	
Biological plausibility of the model	The biological basis of the model construction is questionable due to the enterohepatic recirculation assumption			
Remarks				

Table 1 - Physiological model parameters

Parameters	Values	References	Coherence with other published values [b]
Body weight, BW (kg)	Study specific	Experimental data	
Cardiac output, QCC (L/h/kg$^{0.75}$)	15.87	Fisher et al. (2011))	
Blood flows (fraction of cardiac output)			
Fat (QFatC)	0.053/0.091[a]	Edginton et al. (2006)	
Liver (QLiverC)	0.24	Fisher et al. (2011)	
Brain (QBrainC)	0.11	Brown et al. (1997)	
Skin (QSkinC)	0.058	Brown et al. (1997)	
Gonads (QGonadC)	0.00054/0.00022[a]	Edginton et al. (2006)	
Richly perfused (QRC)	0.76 – QLiverC – QBrainC		
Slowly perfused (QSC)	0.24 – QFatC – QGonadC – QSkinC		
Tissue volumes (fraction of body weight)			
Plasma (VPlasmaC)	0.0435	Fisher et al. (2011)	
Fat (VFatC)	Calculated	Jackson et al. (2002)	
Liver (VLiverC)	0.026	Brown et al. (1997)	
Brain (VBrainC)	0.02	Brown et al. (1997)	
Skin (VSkinC)	0.0371	Brown et al. (1997)	
Gonads (VGonadC)	0.0007/0.0027[a]	Fisher et al. (2011)	
Richly perfused (VRC)	0.33 – VLiverC – VBrainC		
Slowly perfused (VSC)	0.60 – VFatC – VSkinC – VGonadC		

[a] male/female

[b] It would be most useful to have a human physiological parameters database for evaluation of the PBPK models

Table 2 - Chemical specific parameters

Parameters	Values	References	Level of Confidence attributed to the value according to method for determination [1]
BPA			
Hepatic glucuronidation			
Kmliver (nM)	45,800	Coughlin et al. (2012) experimentally determined (pooled male & female human liver microsomes)	**high**
VmaxliverC (nmol/h/kg$^{0.75}$)	707,537	Coughlin et al. (2012) in vitro determination	**high**
Hepatic sulfation			
Kmlivers (nM)	10,100	Kurebayashi et al. (2010)	**high**

Parameters	Values	References	Level of Confidence attributed to the value according to method for determination [1]
		experimentally determined (cryopreserved human hepatocytes)	
VmaxliversC (nmol/h/kg$^{0.75}$)	11,657	Kurebayashi et al. (2010) in vitro determination	**high**
Gastric emptying (GEC, L/h/kg$^{-0.25}$)	3.5	Fisher et al. (2011), Kortejarvi et al. (2007)	**high**
Oral uptake, from small intestine to liver (K1C, L/h/kg$^{-0.25}$)	2[a]	Optimize	**medium**
Glucuronidation in enterocytes			
KmgutC (nM)	58,400	Trdan Lusin et al. (2012) experimentally determined (human intestinal microsomes)	**high**
VmaxgutC (nmol/h/kg$^{0.75}$)	22,750	Trdan Lusin et al. (2012) in vitro determination	**high**
Urinary excretion (KurinebpaC, L/h/kg$^{0.75}$)	0.06	Optimize	**medium**
BPAG			
Uptake from enterocytes into the liver (KGIinC, L/h/kg$^{-0.25}$)	50	Visual fit	**medium**
Volume of distribution (VbodyC, fraction of body weight)	0.0435	Set to plasma volume (Fisher et al., 2011)	medium
Fraction of BPAG in the liver delivered to systemic circulation (MET)	0.9	Teeguarden et al. (2005)	**high**
Urinary excretion (KurineC, L/h/kg$^{0.75}$)	0.35	Optimize	**medium**
Enterohepatic recirculation (EHR)			
EHR as BPA (Kenterobpa1C, L/h/kg$^{-0.25}$)	0.2	Visual fit	**medium**
EHR as BPAG (EHRrateC, L/h/kg$^{-0.25}$)	0.2	Visual fit	**medium**
BPAS			
Volume of distribution (VbodysC, fraction of body weight)	0.0435	Set to plasma volume (Fisher et al., 2011)	medium
Urinary excretion (KurinebpasC, L/h/kg$^{0.75}$)	0.03	Optimize	**medium**

[1] **Indicative level of confidence attributed to the parameter value, according to its determination method**

High	Data **measured** from in vivo or in vitro studies (animal, human tissues)
medium	Data **estimated** by optimization or curve fitting
low	Data **estimated** by other in silico method (e.g. QSAR)

NB: According to the problem formulation, the level of confidence attributed to the value according to its determination method could change

Table 3 - Partition coefficients

Tissue-serum distribution coefficients for BPA were set to in vivo tissue-serum distribution ratios obtained in adult rats (Fisher et al., 2011)

Tissues	Partition coefficients (tissue/serum)	Method for obtention	Level of Confidence attributed to the value according to method for determination [1]
Fat (Pfat)	5.0	in vivo obtained in adult rats	High
Brain (Pbrain)	2.8	in vivo obtained in adult rats	High
Richly perfused tissues (set to brain) (Prich)	2.8	in vivo obtained in adult rats	High
Slowly perfused tissues (set to muscle) (Pslow)	2.7	in vivo obtained in adult rats	High
Gonads (Pgonads)	2.6	in vivo obtained in adult rats	High
Skin (Pskin)	5.7	calculated with algorithm	medium
Liver (Pliver)	0.73	in vivo obtained in adult rats	High

[1] **Indicative level of confidence attributed to the parameter value, according to its determination method**

High	Data **measured** from in vivo or in vitro studies (animal, human tissues)
medium	Data **estimated** by optimization or curve fitting
low	Data **estimated** by other in silico method (e.g. QSAR)

NB: According to the problem formulation, the level of confidence attributed to the value according to its determination method could change

2/ Parameter evaluation and model analysis

Parameter verification and analysis				
Type of information	**Should contain**	**Answer (to be filled in)**	**Comments**	**Suggestion for model improvement**
Model evaluation				
Required information	Prediction of the selected dose metrics and ratio of dose metric prediction towards observed parameters NB: according to the IPCS guidance, the dose metric prediction must be within 2 fold of observed parameters	Publications used for the model evaluation: * Thayer et al (2015) : N = 3 subjects, single oral dose (100 µg/kg BPA in cookie) Good prediction for : - serum BPA, BPAG, BPAS - BPAG, BPAG in urine * Volkel et al (2002) : N = 6 subjects, single oral dose (5 mg BPA in hard-gelatin capsule) Good prediction for : - cumulative excretion of BPAG in urine - plasma BPAG for the first 4h	Prediction in general in line with experimental data (for Volkel 2002 and 2005) Data from Teeguarden et al (2015) were used to optimize the oral uptake constant	Oral uptake of BPA may differ depending on the oral dosing vehicles (cookie versus soup) and/or fasting conditions → studies are needed to understand the impact of dosing vehicles and fasting conditions on BPA kinetics (to reduce uncertainty

		* Volkel et al (2005) : N = 6 subjects, single oral dose (25 µg of BPA in 50ml water) Good prediction for: - cumulative excretion of BPAG in urine * Teeguarden et al (2015): N= 10 subjects, 30 µg/kg BPA (in soup) over-prediction of serum BPA → oral uptake rate constant (K1C) reduced (value obtained by optimization) Good prediction of revised model for: - serum BPA, BPAG, BPAS - cumulative excretion of BPAG in urine	(revised model)	in estimated BPA parameters)
Model Analysis				
sensitivity analysis performed for all parameters	Indication on whether the global sensitivity was performed (if not, must be performed in the next step) Specification on the mode used for the sensitivity analysis: time history or final value mode	See Table 4 A local sensitivity analysis was implemented, with calculation of the normalized sensitivity coefficient (NSC) for 1% increase of the parameter value		The sensitivity analysis should be performed at 2 different concentrations
Uncertainty analysis performed for the most influential parameters	Indication on whether the uncertainty analysis was performed (if not, must be performed in the next step) Specification on the mode used for the uncertainty analysis: time history or final value mode	Monte Carlo simulations were conducted to evaluate the inter-individual variability of model predicted internal dose metrics (C_{max} and daily AUC) of serum BPA with different exposure scenarios (global uncertainty analysis) Predicted percentiles of the distribution of serum BPA dose metrics are indicated, however individual uncertainty analysis (specially on sensitive parameters) has to be performed thanks to the P95 and P50 values	Performed with oral uptake constant (K1C), determined based on the cookie data (Thayer et al, 2015)	

Table 4 - Sensitive model parameters (Parameters with absolute NSC values greater than 1 are highlighted in bold)

Physiological parameters	BW, **QCC**, QLiverC, QFatC, **QRC**, **QSC**, VliverC, VfatC, VRC, VSC
Partition coefficients	Pfat, Prich, Pslow, Pliver
Chemical specific model parameters	**Kmliver**, **VmaxliverC**, Kmlivers, VmaxliversC, **GEC**, **K1C**, KmgutC, **VmaxgutC**, **MET**, Kenterobpa1C, EHRrateC

3/ Conclusion on the refinements needs for this BPA model

Conclusion

This PBPK model can reproduce the BPA chemical-specific pharmacokinetic data for oral exposure though solid form (cookie) and is reliable with regard to its predictions of BPA in serum (Thayer et al 2015, N=3 volunteers), BPAG in serum (Thayer et al, N=3 volunteers), cumulative excretion of BPAG in urine (Thayer et al 2015, N=3 volunteers and Volkel et al 2002, 2005).

Needs for refinement:

For oral exposure though liquid form (soup), the PBPK model has been revised (re-calibrated by optimization of the oral uptake constant) however not evaluated with new data.

Uncertainty analysis would have to be performed with concentrations of urinary BPA, urinary BPAG and serum BPAG at 24h.

The model should be evaluated further, in particular towards the biological relevance of the enterohepatic recirculation modelisation.