Case study illustrating the WHO IPCS guidance on characterization and application of physiologically based pharmacokinetic models in risk assessment

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Abstract
The World Health Organization (WHO) International Programme on Chemical Safety (IPCS) guidance on Characterization and Application of Physiologically Based Pharmacokinetic Models in Risk Assessment (IPCS, 2010) describes key principles for risk assessors and model developers. In the WHO Guidance, a template for model documentation was developed and a case study included. Here the WHO Guidance, including the template, is summarized and an additional case study is presented to illustrate its application, based upon an existing risk assessment for 2-butoxyethanol (CAS NO. 111-76-2). The goal of the WHO Guidance and the current paper is to increase regulatory acceptance of complex biologically descriptive pharmacokinetic (or toxicokinetic) models, such as PBPK models, by facilitating communication and successful interaction between modelers and risk assessors.

Keywords: Risk assessment Physiologically based pharmacokinetic (PBPK) modeling 2-Butoxyethanol

1. Introduction


PBPK models are quantitative descriptions of the absorption, distribution, metabolism, and excretion (ADME) of chemicals in biota based on interrelationships among physiological, biochemical, and physicochemical determinants of these processes [Teorell, 1937a,b; Dedrick et al., 1972; Fiserova-Bergerova, 1983; NRC, 1987; Andersen, 2003; Reddy et al., 2005; Lipscomb and Ohanian, 2007]. These models fit within the broader continuum of increasingly data-informed approaches for risk assessment, ranging from the commonly adopted defaults based on external dose to more biologically informed or realistic internal dose–response models. By facilitating the incorporation of internal dose measures appropriate to the mode of action (MOA) by which a chemical is hypothesized to cause a critical toxic effect, PBPK models facilitate more scientifically sound extrapolations across studies, species, routes, and dose levels (Clewell and Andersen, 1985, 1987; Clewell et al., 2002; Clark et al., 2004; Chiu et al., 2007; Loizou et al., 2008; Thompson et al., 2009). These models are also fundamental to the development of biologically based dose-response models and to addressing uncertainty and variability related to toxicokinetics (TK) and toxicodynamics.

The WHO Guidance is a product of an initiative undertaken as part of the IPCS Project on Harmonization of Approaches to the Assessment of Risk from Exposure to Chemicals. It complements the outputs of other initiatives of this Project, particularly those relating to the weight of evidence for MOA of chemicals, human exposure models, and chemical-specific adjustment factors (CSAFs) (IPCS, 1999, 2004, 2005a,b, 2008; Sonich-Mullin et al., 2001; Meek et al., 2003; Boobis et al., 2006, 2008). The WHO Guidance focuses on best practice for characterizing and applying PBPK...
models, to facilitate their uptake by the regulatory community as exemplified recently for pharmaceuticals (Zhao et al., 2012).

Additionally, capitalizing on initiatives within the USA, Canada and Europe (Gentry et al., 2004; U.S. EPA, 2006; Barton et al., 2007; Loizou et al., 2008), the WHO Guidance provides: (1) an international perspective, (2) a basis for international endorsement of the best practices for characterizing and applying PBPK models in risk assessment, and (3) a means of raising the level of awareness on the applicability and value of PBPK modelling in risk assessment.

The WHO Guidance documents key principles both for risk assessors who need to evaluate PBPK models as well as for PBPK model developers (referred to as PBPK modellers) who are interested in developing models for application in risk assessment. It includes, for example, a checklist of characteristics for selecting PBPK models for use in risk assessment (Table 1).

The WHO Guidance emphasizes the need for effective and transparent communication, which is central to facilitating practical application of PBPK models in risk assessment. Presentation of key model parameters and critical aspects of the models is important not only in increasing the understanding of model development and applicability by risk assessors; it is also essential to gaining regulatory acceptance. Development of model descriptions in a standard format to effectively transfer information about the model, the data supporting it and the appropriateness of its application under a range of conditions, is an important component contributing to increased communication. To this end, a template for PBPK model description was presented in the WHO Guidance and is reproduced below (Fig. 1).

The principal objective of this manuscript is to provide an additional case study, to illustrate use of this template. The case study addresses 2-butoxyethanol (BE) based on an assessment conducted under the Canadian Environmental Protection Act (Environment Canada and Health Canada, 2002, 2003). Since the objective is to provide additional illustration of the WHO Guidance, rather than to consider the specific chemical, per se, consideration of available data on BE is restricted to that cited within the Health Canada assessment and supporting documentation. Similarly, the approach described in the case study is that presented in the assessment; it has not been additionally vetted or revised to reflect, for example, potentially impacting methodological advances. It is also restricted to consideration of the critical endpoint for which PBPK modeling was applied.

2. Documenting a PBPK model for risk assessment

According to the WHO Guidance, in order to facilitate transparency, reproducibility, and credibility, while the extent of documentation of a PBPK model intended for use in risk assessment may vary depending on end use, it requires the inclusion of
sufficient information to enable accurate reproduction and evaluation of model performance by an experienced modeller. Key in this evaluation is to address whether the available model is fit for the proposed assessment or whether further modifications are required. This requires that modellers provide enough details to facilitate a clear understanding to enable reproduction of the input–output relationships for the dose metric of relevance to MOA [examples are area under the plasma or blood concentration versus time curve (AUC) or maximal concentration (Cmax) of parent or a metabolite, or formation rate of a reactive metabolite]. Overall, PBPK model documentation should address the following broad topics:

- Scope and purpose of the model.
- Model structure and biological characterization.
- Mathematical description of ADME.
- Computer implementation and verification.
- Parameter estimation and analysis.
- Model validation and evaluation.
- Evaluation/justification of dose metrics.
- Specialized analysis, if any (e.g., population variability).

As is described in the WHO Guidance, the above aspects can be captured in summary form for the risk assessment audience but in greater depth for specialists, as is typically done in technical publications. In the latter case, it is particularly important to identify clearly the data sets that were used to evaluate the model, along with the rationale for excluding data sets, if any, during model development. Similarly, the alternative model structures considered, the range of values assigned for each of the input parameters as well as the exposure conditions for sensitivity, uncertainty, and variability analyses should be presented along with the rationale. For risk assessment application, the original model code, corresponding to the published manuscript, is essential and should be provided to the regulatory scientists for independent evaluation and reproduction of any simulations that form the basis of dose metrics used in the risk assessment. Supporting files and data sets sufficient to reproduce published plots (comparing the model simulations with the experimental data) and reported numerical results (exposure/dose calculations) should also be submitted to the regulatory scientists. Further justification of the dose metric on the basis of plausibility and consistency with available information on MOA as well as dose–response calculations for the critical effect should also be presented and necessarily requires input from a range of multidisciplinary experts (see IPCS, 2005a).

3. Template for documentation of a PBPK model for risk assessment

Presented here is information relevant for description of a PBPK model for application in chemical risk assessment, as developed in the WHO Guidance (IPCS, 2010). Four areas are included in the template: (1) background on the chemical, its PK and MOA, (2) characterization and evaluation of the PBPK model, (3) modelling and evaluation of the model-derived dose metrics, and (4) PBPK modelling and comparison with the default assessment. The brief descriptions of the sections included here should be read in conjunction with the more complete explanations included in the WHO Guidance document and the BE case study presented subsequently.

3.1. Background

This section describes the overall objective of the risk assessment (e.g., deriving a guidance value for the inhalation route based on a cancer bioassay conducted in rats). It also specifies the objective of the internal dose–response analysis for which the PBPK model is relevant (e.g., to calculate the human-equivalent exposure concentration of a chemical that yields the same level of dose metric that produces a given level of effect in the responding test species).

3.1.1. Critical effect

A summary of the toxicological database on the chemical is presented with emphasis on the target organs and tissues and the observed effects. Information on dose–response is described with focus on the critical studies. Evaluation of multiple endpoints will often be appropriate when applying MOA and pharmacokinetics in dose–response analyses because it can be difficult to determine in advance which endpoints will be estimated to have the greatest risk or lowest health protective concentrations.

3.1.2. Pharmacokinetics

Pathways and processes involved in the absorption, distribution, metabolism, and excretion of the chemical are described, including a metabolic scheme showing the different pathways and metabolites (particularly the potential dose metrics). Dose dependency, species- and route-specific observations as well as the role of genetic or phenotypic variants (e.g., polymorphisms of enzymes or transporters), to the extent they are known, are emphasized.

3.1.3. Mode of action (MOA)/relevant dose metric

The choice of the appropriate dose metric is based upon its demonstrated relevance to MOA. This necessitates clear delineation of key events in the hypothesized mode of action (see suggested schematic presentation in Fig. 2) and systematic analysis of the supporting weight of evidence. This analysis is based on widely accepted Bradford Hill considerations of dose-response as well as temporal concordance of key events, strength, consistency, specificity and biological plausibility (i.e., reflective of physiological reality and consistent with the current state of knowledge).
Table 2
Confidence in the use of plausible dose metrics for a chemical based on an understanding of MOA for the critical effect (N, none; L, low; M, medium; H, high) (IPCS, 2010).

<table>
<thead>
<tr>
<th>Dose metric options</th>
<th>Confidence based on MOA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposure concentration of parent chemical</td>
<td>N(^a)</td>
</tr>
<tr>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Model-derived dose metric(^{b,c})</td>
<td>??(^b)</td>
</tr>
</tbody>
</table>

\(^a\) Examples: AUC or C\(_{\text{max}}\) of parent or metabolite and rate of formation of a metabolite.
\(^b\) These items in the table would be filled in for a specific example.

(Meeke et al., 2003; Seed et al., 2005; Boobis et al., 2006, 2008; Meek, 2008; Meek and Klaunig, 2010). Relevant evidence includes results from in vitro and in vivo studies relating to the key role of the parent chemical, a specific metabolite, or a metabolic pathway in the toxicity in test animals and humans. Supporting data may include observations from studies with genetically altered mice, depletion of cofactors (e.g., glutathione [GSH]), enzyme induction, inhibition, toxicity of metabolites, etc.

Based on the review of the information on MOA for each critical effect, the confidence associated with each of the plausible dose metrics may be summarized, as in Table 2. These degrees of confidence are generally specified based on comparisons with MOA analyses for other substances. They take into account the quantity of supporting information along with quality, consistency, specificity, and biological plausibility.

3.1.4. Scope for PBPK model application

The proposed strategy for use of the PBPK modelling results in risk assessment is described. For example, the PBPK model application might address the following question:

What is the human-equivalent concentration of a risk-specific inhalation exposure in rodents, on the basis of an equivalent dose metric at the target tissue?

The strategy for making the interspecies comparisons would be stated (e.g., based on measures of central tendency, such as means or medians). Also, it would be indicated whether application of the PBPK model addresses the interspecies differences in PK only, or PK and PD. This is critically important as a basis for appropriate application of factors to address the remaining elements of uncertainty for CSAFs (IPCS, 2005a).

3.2. PBPK model: characterization and evaluation

3.2.1. Model capability and selection

In this section, the capability of the selected PBPK model(s) to simulate dose metrics in the needed species, sex, life stage, and exposure route (e.g., adult male rats and adult humans exposed by inhalation) is reviewed. Also, it is essential to describe whether the chosen model is capable of simulating all or only some of the candidate dose metrics identified in Table 2. The rationale for choosing one model over others available is addressed.

3.2.2. Model structure and biological characterization

The model is described in terms of physiological compartments and biological processes. Rationale for the choice of specific physiological compartments is included (e.g., is the structure similar to that of models established for other chemicals or chemical classes?) A schematic representation of the model that clearly indicates the extent to which the metabolic scheme is integrated within the model is also included (e.g., Fig. 3). The routes of absorp-

3.2.3. Parameter estimation and analysis

The sources from which the parameter values were obtained are described. This could include derivation of model parameters such as partition coefficients and metabolism constants on the basis of iterative fitting to in vivo data (e.g., plasma time course, total metabolism by gas uptake, GSH depletion, urinary metabolite levels), in vitro data or in silico methods (Ramsey and Andersen, 1984; Clewell and Andersen, 1985; Krishnan and Andersen, 2007). Justification is included for the use of in silico methods (e.g., in relation to applicability domain), allometric scaling and in vitro–in vivo scaling of parameters, if applicable. When the parameter is not directly available in the cited reference source, but calculated from data reported in that source, the manner of derivation of the specific values obtained for use in the model is described.

Key model parameters having significant influence on the dose metric predictions (model outcome) for both test animals and humans are provided, preferably in tabular form. Local analysis is a commonly applied method to identify key parameters, though global sensitivity analysis or other methods may also be used. Where possible, the complete list of all model parameters should be appended to the document along with their values and sources. Even though all parameters may not be critical to the dose metric prediction in humans and test animals, some are likely to be relevant to model simulation and parameter estimation in other studies or for other purposes (e.g., exposure routes not in the current risk assessment).

3.2.4. Purpose-specific model evaluation

The evaluation of the model is described, focusing on the level of confidence in its structure and parameters as well as its adequacy to predict the dose metrics for the intended risk assessment application. The purpose specific reliability of the PBPK model for

![Fig. 3. Structure of the PBPK model for a volatile organic chemical.](image-url)
conducting, for example, interspecies extrapolation of PK is described.

A summary list of experimental data/studies that were compared with the simulations of the PBPK model is provided. Based on simulation outputs, it is indicated whether the model reproduces the shape of the time course PK data in experimental animals (and, if available, humans). The ability of the model to consistently reproduce the general trend of the data (i.e., peaks, bumps and valleys, saturation of metabolism) or only portions of one or more data sets is addressed. Proximity of the model simulations to the experimental data (e.g., are they within a factor of two on average) is also discussed, recognizing that the experimental data in reality constitute only one sample of the hypothetically plausible range of values. Sensitivity analyses can characterize to which parameters in the model predictions are most sensitive, thus indicating those parameters for which greatest confidence and documentation is desirable. Ideally, good consistency between pharmacokinetic data and model predictions would be observed for data sensitive to these key parameters.

The level of confidence in the PBPK model for its predictions of dose metrics intended for the risk assessment purpose (e.g., interspecies extrapolation of PK) is established on the basis of the following considerations:

- **Biological basis:** do the model structure and parameters have a reasonable biological basis?
- **Model simulations of data:** how well does the PBPK model reproduce the chemical-specific TK data under various experimental or exposure conditions?
- **Reliability (model testing, uncertainty, and sensitivity):** how reliable is the PBPK model with regard to its predictions of dose metrics relevant to risk assessment?

The reliability of the model predictions of dose metrics for the risk assessment is described, where feasible, 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 is questionable. The results may be presented in summary form, as illustrated in Fig. 4. The values describing low, medium, and high uncertainty or sensitivity should be assigned on a case-by-case basis, depending upon, among other things, the expected distribution of the variables considered.

Although the scaling of this analysis is subjectively defined, the qualitative overview of sensitivity and uncertainty conveys the expected behaviour of the model and parameter confidence by considering:

- For the human risk assessment conditions (e.g., exposure pathways, relevant exposure conditions such as acute or chronic doses or concentrations at either the human equivalent dose or concentration or a proposed guidance value), which parameters most strongly influence the dose metric; and
- For the study or studies from which the critical end-points are derived (i.e., toxicity, epidemiological, clinical studies), which are the model parameters to which the dose metric predictions are most sensitive.
- For pharmacokinetic data used either for model evaluation or parameter estimation, to which parameters sensitivity analysis indicates these data are most informative.

### 3.2.5. Model documentation

This section addresses how the model was implemented and which simulation languages were used. Whether or not the model code is available is indicated, as is the availability of the numerical values for all model parameters along with distributions of parameters used for variability/uncertainty analysis, if applicable.

#### 3.2.6. Model peer review

In this section, the nature of publication (e.g., in the peer-reviewed literature) is addressed as are any further independent review of the models and codes, for example, by regulatory agencies for specific risk assessment applications. The extent and nature of these peer reviews is described, with the outcome summarized.

#### 3.3. PBPK modelling and evaluation of dose metrics

The dose metrics computed with the PBPK model for the point of departure for a particular critical endpoint (e.g., no-observed-adverse-effect level) or the dose metrics associated with each of the treatment groups (e.g., for benchmark dose analysis) are presented. The relationships between the various dose metrics and responses observed in critical studies are described, with consideration of the basis for choosing among alternative dose metrics and specific doses.

The confidence in the choice of the dose metric is evaluated based on the reliability of model predictions and consideration of the dose–response information for the endpoint of concern in the key studies as well as any other available relevant studies, while acknowledging differences in exposure scenario, route, and species. These observations can be summarized as in Table 3 to provide comparisons of the level of confidence in alternative dose metrics, on the basis of both MOA as well as PBPK modelling. The summary of levels of confidence in the various dose metrics allows the assessor to choose the appropriate dose metrics for the

<table>
<thead>
<tr>
<th>Dose metric options</th>
<th>Confidence based on MOA</th>
<th>Confidence in simulation based on PBPK modelling</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposure concentration of parent chemical</td>
<td>N*</td>
<td>N*</td>
</tr>
<tr>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Model-derived dose metric</td>
<td>??? a</td>
<td>??? a</td>
</tr>
</tbody>
</table>

* These items in the table would be filled in for a specific example.
assessment that would be consistent with the nature of the assessment, understanding fully the tradeoffs and level of effort required.

The results of dose-response modeling based on the dose metrics generated using the PBPK models (in lieu of exposure concentrations or exposure doses) is also presented in this section, to conduct various extrapolations for deriving the human exposure values.

3.4. PBPK model application and comparison with default

The results of default analyses relevant to the particular assessment are presented. This facilitates the evaluation of the value of PBPK modelling as well as placing it in the context of its contribution to reducing relative uncertainty in PK in a specific risk assessment. It is addressed based on considerations of the: (1) choice of dose metrics, (2) conceptual model, and (3) input parameters (Fig. 5). When the pharmacokinetics are nonlinear, the ordering of steps in the derivation of a toxicity reference value can have an impact on the resulting value so, careful evaluation of different approaches may clarify sources of differences among default and PBPK-derived values.

4. Case study—chemical 2-butoxyethanol (BE)

This case study reviews relevant data as a basis to reduce uncertainty (compared to default approaches) through quantification of interspecies differences in toxicokinetics, using PBPK. The case study addresses BE based on an assessment conducted under the Canadian Environmental Protection Act (Environment Canada and Health Canada 2002, 2003). Since the objective is to provide additional illustration of the WHO Guidance (and associated template for presenting PBPK models for risk assessment), rather than to focus on the specific chemical, per se, consideration of available data on BE is restricted to that cited within the Health Canada assessment and supporting documentation. In addition, the assessment refers to three modestly different versions of the PBPK model (Corley et al., 1994, 1997; Lee et al., 1998, but this case study will consider them to be effectively one model and not address the minor differences between them since they all fit reasonably well to the same human kinetic data for the inhalation route (Environment Canada and Health Canada 2002). Ideally, either one model would be used in the risk assessment or if multiple models were used, the basis of that choice would be explained and the differences clearly described.

4.1. Background

BE is a volatile chemical for which the risk assessment establishes a guideline value (e.g., threshold concentration for humans) in air as a basis for comparison with estimated exposure, for which the principal route is inhalation.

4.1.1. Critical effect

The critical effects of BE are those on the hematological system (reviewed in Environment Canada and Health Canada 2002, 2003). Specifically, BE causes alterations of hematological parameters characteristic of hemolytic anemia, hemoglobinuria and increased fragility of erythrocytes following short-term or long-term exposure via inhalation, ingestion or dermal contact in rats, mice, rabbits, dogs, and monkeys.

The critical toxicological study involved the exposure of 50 male or female F344 rats per group to concentrations of 0, 31.2, 62.5, or 125 ppm for 6 h per day for two years (NTP, 1998). Hematological parameters measured in 10 rats per group per time point indicated that there were significant changes at the lowest exposure levels; there was also some indication of concentration-related trend for various hematological parameters.

Other endpoints were observed in the toxicological studies, including effects in the forestomach, but these were not selected as the critical effect for the risk assessment analysis using PBPK modeling.

4.1.2. Pharmacokinetics

The data on metabolism and toxicokinetics of BE are summarized by Environment Canada and Health Canada (2002, 2003).

Briefly, BE is absorbed and distributed to tissues following
inhalation, oral, and dermal exposures. It is metabolized by alcohol/aldehyde dehydrogenase in the liver to an acetic acid metabolite, butoxyacetate, referred to hereafter as BE-M, and by CYP2E1 to butyraldehyde and ethylene glycol via O-dealkylation (Fig. 6).

4.1.3. Mode of action/relevant dose metric

The proposed key events in the hypothesized mode of induction of BE toxicity are indicated below and presented in Fig. 7:

- Oxidative metabolism to the acetic acid metabolite (BE-M).
- Conjugation of BE-M with membrane lipids with resulting increases in permeability.
- Erythrocyte swelling and lysis.
- Decreased erythrocyte count, hemoglobin, and hematocrit, and in response, increased production of immature erythrocytes (reticulocytes) by the bone marrow.

The substantial database supporting the MOA, for which the weight of evidence is considerable, also satisfies criteria of concordance of dose response and temporality for subsequent key events/critical effect and relevant dose metric as well as strength, consistency, specificity, and biological plausibility. In relation to consistency, in vitro studies with BE-M found it far more potent than the parent chemical, BE, in inducing haemolysis. Sensitivity to BE-induced hematologic effects is correlated with variations in production and clearance of BE-M; inhibitors of alcohol and aldehyde dehydrogenases that metabolize BE to BE-M, reduced significantly the hematologic effects associated with BE exposures in rats.

The acetic acid metabolite is more effective than the parent chemical in causing hemorrhagic effects on the erythrocyte membranes and associated effects. In vitro studies have shown that BE-M at concentrations of 0.5–2 mM resulted in increases in hematocrit followed by hemolysis; however, hemolysis was not induced by the parent chemical at concentrations up to 10 mM in the in vitro systems (Ghanayem 1989).

Given that the available evidence suggests a key role for metabolism in the mode of action, a measure of internal exposure to BE-M (e.g., Cmax, AUC in blood) would appear to be the appropriate dose metric.

Based on the review of the information on MOA for the critical effect, the confidence associated with each of the plausible dose metrics is summarized in Table 4.

<table>
<thead>
<tr>
<th>Dose metric options</th>
<th>Confidence based on MOA</th>
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</thead>
<tbody>
<tr>
<td>Exposure concentration of parent chemical</td>
<td>N</td>
</tr>
<tr>
<td>Blood concentration of parent chemical</td>
<td>L</td>
</tr>
<tr>
<td>Blood concentration of active metabolite</td>
<td>H</td>
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</tbody>
</table>
4.1.4. Scope for PBPK model application

Adequate experimental data on candidate dose metrics of relevance to the MOA of BE, both in rats and humans, essential for computing the magnitude of $AK_{AF}$ (i.e., the assessment factor reflective of the uncertainty in rat to human difference in toxicokinetics) are not available. Specifically, the $C_{\text{max}}$ and AUC of BE-M in each of the dose groups of the critical toxicity study in rats reported above were not adequately determined. For example, in the critical study, the $AUC_{\text{BE-M}}$ was reported only for the postexposure period (i.e., the AUC during the exposure period was not known). So, the rat PBPK model could be used to simulate the dose metrics of relevance to the mode of action of BE (i.e., $C_{\text{max}},$ AUC of BE-M) for the various dose levels used in the critical study. Total AUC (i.e., during exposure + postexposure) in the test animals can be estimated based on the model.

There is also a limitation regarding the available human data for BE. In a single relevant experimental study, five human male subjects were exposed by inhalation to 20 ppm for a 2-h period of light physical exercise (50 W), and the BE-M levels were determined in venous blood samples at 0, 2, 4, and 6 h from the start of the exposure. In this case, data on human $AUC_{\text{BE-M}}$ required for calculating $AK_{AF}$ were collected under exercising conditions. These might not be reflective of the kinetics under resting conditions, because the respiratory uptake of BE is linearly related to the ventilation rate. Therefore, the correction of AUC value in the available human study on the basis of physiological conditions during working versus resting conditions could be conducted using a human PBPK model. Thus, the use of a PBPK model would facilitate the incorporation of better and more relevant data for calculation of $AK_{AF}$ in the risk assessment of BE.

4.2. PBPK model: characterization and evaluation

4.2.1. Model capability and selection

The critical toxicological studies were conducted in rats exposed by the inhalation route. The focus of the risk assessment is to develop a guideline value for BE in air as a basis for comparison with exposure of the general population, for whom inhalation is the principal route. Therefore, PBPK models were selected that were capable of simulating potential dose metrics, particularly BE and BE-M in blood, in both adult rats (test animals) and adult humans exposed to this chemical by inhalation.

4.2.2. Model structure and biological characterization

The PBPK model consisted of two submodels, one for the parent chemical BE and another for the toxic metabolite BE-M. Each submodel in the Corley et al. (1994) model consisted of the following compartments: lung, gastrointestinal tract and liver, skin, muscle, fat, rapidly perfused tissues, and other slowly perfused tissues, interconnected by blood circulation [with modest variations of compartments in the Lee et al. (1998) model]. The kidney was represented additionally as a separate compartment in the metabolite

![Diagram of PBPK model](image-url)
model. The input of BE into the system was through inhalation, intravenous infusion or oral dosing (gavage or drinking water) whereas the input for the BE-M model was the flux through the metabolic pathway producing BE-M in the liver. The metabolism of BE in liver, clearance of BE via exhalation and elimination of BE-M via urine were represented as shown in the rat and human models in Fig. 8.

4.2.3. Parameter estimation and analysis

The physiological parameters for the rat and human PBPK models were obtained from published literature. The blood:saline partition coefficients for both species (i.e., rat, human) as well as rat tissue:saline partition coefficients (for muscle, stomach, small intestine, cecum, lung, skin, kidney, and fat) for BE and BE-M were determined by ultrafiltration. The maximal enzymatic velocity (Vmax) and half saturation concentration (Km) to describe the saturable metabolism of BE (mediated by alcohol/aryl hydrocarbon hydroxylase) were obtained from perfused liver experiments in the rat, and Vmax was scaled to humans on the basis of body weight0.7. The protein binding constants were arbitrarily set equal to the molar equivalent values for phenolsulphonphthalein and considered to be species invariant. Sample values as well as the estimation methods or sources of input parameters of the rat and human BE PBPK models are listed in Tables A.1 and A.2 (see Appendix A).

4.2.4. Purpose-specific model evaluation

The experimental data in rats and humans that were simulated by the PBPK model are summarized in Table 5 and Appendix B (Figs. B2–B4). Visual inspection suggests that the model is capable of simulating the shape of the time-course PK data in rats and humans. Figs. B2–B4 indicate that the model consistently reproduces the general trend of the data rather than just portions of one or more datasets. Further, the model simulations are visually close (i.e., within a factor of two on average) to the experimental data. Since the animals (or humans) used in estimating parameters by fitting to the data versus those used in generating the various TK data for model evaluation purposes are not the same, some level of discordance is expected. In the specific case of BE, the PBPK model simulations were within a factor of two of the experimental data on respiratory uptake of BE, urinary levels of BE-M following a 6-h inhalation exposure to 4.2–438 ppm BE as well as urinary concentrations of BE-M after inhalation exposure of rats for 12 days to 20–100 ppm BE. The human model simulations were also within a factor of two of blood concentrations of BE following 2-h inhalation exposure to 20 ppm in exercising individuals. The model simulation of the time course of the blood concentration of BE-M following inhalation exposures is directly relevant to the potential dose metrics (Cmax, AUC) for the risk assessment.

The data above on the results of model simulation are considered in the context of the following questions, as outlined in the template for documentation:

- **Biological basis**—Do the PBPK model structure and its parameters have a reasonable biologically basis?
- **Model performance**—How well does the PBPK model reproduce the chemical-specific TK data under various experimental or exposure conditions?
- **Reliability**—How reliable is the PBPK model with regard to its predictions of dose metrics relevant to risk assessment?

Based on biological basis, model performance, and reliability of dose metric predictions, there is a high level of confidence in the PBPK model for the conduct of interspecies extrapolation (rat to human) using dose metrics related to BE (Table 6).

For purposes of this case study, the limited sensitivity results presented in Lee et al. (1998) were used to consider parameter uncertainty and sensitivity (Fig. 9). These authors evaluated a subset of parameters at two concentrations (31.2 and 1250 ppm). At a high concentration saturating for metabolism of BE to form BE-M, Km1 (referring specifically to the metabolism of BE to BE-M) has low sensitivity, while both Vmax and Km show sensitivity at the low concentration. The model predictions of blood BE-M concentrations were sensitive towards the Vmax and Km of metabolic processes (essentially in opposite direction to those observed for the parent chemical, BE). Further the plasma protein binding parameters were also highly sensitive. Uncertainty in the parameters was qualitatively assessed considering the available data used to estimate the parameters. A similar analysis for the human model under the exposure conditions relevant to the human risk assessment would also normally be reported for the template, but was not available to include here.

### 4.2.5. Model documentation

The model was written and solved in SimuSolv™. Parameter optimization was also conducted using this software. The model equations, that are different from previously published PBPK models, are listed in the appendix of the publications (Corley et al., 1994; Lee et al., 1998). All parameter values, specific to the species were listed in tabular form. The details of the parameter estimation methods were provided in the Methods section of the publications (Corley et al., 1994; Lee et al., 1998). (See Appendix A for parameter

### Table 5

Data used in the evaluation of the PBPK model for BE (Corley et al., 1994).

<table>
<thead>
<tr>
<th>Species</th>
<th>Gender</th>
<th>Dose</th>
<th>Route</th>
<th>Data</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat F344</td>
<td>Male</td>
<td>31.25, 62.5, and 125 (mg/kg)</td>
<td>Intravenous</td>
<td>BE and BE-M blood concentration</td>
<td>Ghanayem et al. (1990)</td>
</tr>
<tr>
<td>Rat F344</td>
<td>Male</td>
<td>4.2, 49, and 438 ppm</td>
<td>Inhalation (nose-only)</td>
<td>Respiratory uptake of BE, amount of BE in urine, and total amount metabolized</td>
<td>Sabourin et al. (1992)</td>
</tr>
<tr>
<td>Rat F344</td>
<td>Male</td>
<td>8.6 and 126 (mg/kg)</td>
<td>Oral</td>
<td>BE and BE-M blood concentration at 3, 6, 12, and 24 h</td>
<td>Corley et al. (1994)</td>
</tr>
<tr>
<td>Rat F344</td>
<td>Male</td>
<td>125 and 500 (mg/kg)</td>
<td>Oral</td>
<td>Amount of BE-M in urine</td>
<td>Ghanayem et al. (1987a,b)</td>
</tr>
<tr>
<td>Rat F344</td>
<td>Male</td>
<td>28, 47, and 140 mg/kg (290, 860, and 2590 ppm in drinking water)</td>
<td>Oral</td>
<td>Amount of BE-M in urine (3.7, 7.3, and 16.1 mg) and BE metabolized 72 h following the start of exposure (5.2, 10.8, and 27.5 mg eq.)</td>
<td>Medinsky et al. (1990)</td>
</tr>
<tr>
<td>Human</td>
<td>2-h exposure; 20 ppm</td>
<td>Inhalation with assumption of dermal uptake</td>
<td>BE and BE-M concentration in finger prick blood</td>
<td>Johanson et al. (1986), Johanson and Johnson (1991)</td>
<td></td>
</tr>
<tr>
<td>Human</td>
<td>2-hr exposure; 50 ppm + 2-h exposure, 50 ppm</td>
<td>Inhalation mouth only followed by dermal</td>
<td>BE concentration in venous blood draining the skin</td>
<td>Johanson and Boman (1991)</td>
<td></td>
</tr>
</tbody>
</table>

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4.3. Evaluation of dose metrics

An analysis of the published literature indicates that sensitivity to BE-induced hematologic effects is correlated with variations in production and clearance of BE-M. The acetic acid metabolite, BE-M, is more effective than the parent chemical BE in causing hemolytic effects on the erythrocyte membranes and associated effects. Taken together, the available evidence indicates a key role for BE-M in the toxicity. Therefore, a measure of internal exposure to BE-induced hematologic effects in female rats was chosen as the basis for the point of departure of 5.3 mg/m³ in rats.

The rat PBPK model was applied to adjust an experimental exposure in rats of 62.5 ppm (302 mg/m³) for 6 h to AUC values for the acid metabolite (BE-M) in blood. The result was an AUC value of 2077.5 μM × h, which was concentration normalized (2077.5 μM × h/62.5 ppm/6 h) to an AUC value of 5.54 μM × h/ppm-h. While the relationship between AUC and exposure concentration seems consistent in the range of observations (4.2–438 ppm), it is not clear whether the relationship between AUC and exposure concentration is consistent with decreasing dose in rats. A human PBPK model was developed and applied to working (50 W activity) and resting humans, with a resulting concentration normalized AUC value for BE-M comparable to that obtained from adjustment of ventilation rates for working and resting conditions (2.73 μM × h/ppm-h). The resulting ratio between the AUC for humans and rats is 2.73/5.54, or 0.5. This value serves as the CSAF value for animal to human toxicokinetic differences, AFAK. Applying this CSAF value to the rat duration-adjusted BMCL05 of 5.3 mg/m³ for hematotoxicity yields a human equivalent concentration of 5.3 mg/m³ ÷ 0.5 = 10.6 mg/m³.

4.4.2. Default approaches

Human equivalent concentrations estimated using default methods range from 1.3 to 18.6 mg/m³, depending upon the method. The following presents a continuum of four possible default methods to extrapolate exposures from animals to humans. These approaches are consistent in concept, in that none takes into account the toxicodynamic component of interspecies extrapolation.

<table>
<thead>
<tr>
<th>Aspect</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biological basis</td>
<td>The model parameters, structure, and assumptions are biologically plausible and consistent with available data.</td>
</tr>
<tr>
<td>Performance (data vs. model)</td>
<td>The rat model and the human model, with the chemical-specific and species-specific parameters (Tables A1 and A2) along with calculations of age-specific changes in parameters, simulated TK data from various experiments. The model consistently simulated the TK profile (i.e., bumps and valleys) of BE and its metabolite (BE-M) following inhalation exposure (the route of relevance to the risk assessment) in male rats and humans. The rat model reproduced the blood concentrations of BE and BE-M following iv doses ranging from 31.25 to 125 mg/kg, inhalation exposure from 125 to 312 ppm, and oral doses of 8.6–500 mg/kg. The rat PBPK model also reproduced the respiratory uptake of BE and urinary levels of BE-M following a 6-h inhalation exposure to 4.2–438 ppm BE as well as BE-M urinary concentrations after inhalation exposure of rats for 12 days to 20–100 ppm BE. The human model reproduced the uptake and blood concentrations of BE following 2-h inhalation exposure to 20 ppm in exercising individuals. Additionally, the kinetics of BE and BE-M in blood as well as cumulative excretion of BE-M in humans exposed by inhalation to 50 ppm for 2 h or by dermal route to 50 ppm BE for 2 h was simulated using the PBPK model.</td>
</tr>
<tr>
<td>Reliability of dose metric predictions</td>
<td>The model simulations were compared with empirical data on the toxic moiety reflective of the dose metric (i.e., blood concentrations of BE-M) (1) in rats exposed by inhalation to atmospheric concentrations in the range of the dose–response study (31–250 ppm) as well as (2) in humans exposed to a low concentration (i.e., first order range for metabolism) of relevance to risk assessment.</td>
</tr>
</tbody>
</table>
and that each includes a duration adjustment from intermittent to continuous exposure. From studies with rats, BMCL\textsubscript{05} for hematological effects in terms of BE (parent chemical) in rats was 29.7 mg/m\textsuperscript{3} for 6 h/day, 5 days/week.

The first approach assumes that the toxicokinetics are the same in rats and humans. Only the duration adjustment is applied.

\[
29.7 \text{ mg/m}^3 \times (6 \text{ h/24 h}) \times (5 \text{ days/7 days}) = 5.3 \text{ mg/m}^3
\]  

(1)

In the second of these default approaches, a duration-adjustment from intermittent to continuous exposures, is incorporated in rats and humans, respectively, and applies the default value of 4 for interspecies differences in kinetics (AK\textsubscript{UF}).

\[
5.3 \text{ mg/m}^3 \times 4 = 1.3 \text{ mg/m}^3
\]  

(2)

The third approach addresses the total amount absorbed into the bloodstream, by adjusting exposure duration from intermittent to continuous (as above) and assuming 100% absorption of the inspired concentration in both animals and humans; the species adjustment is made based on amount absorbed per body mass calculated by considering exposure duration adjustment and respiratory volume.

For this approach, default values for breathing rate and body mass are used:

<table>
<thead>
<tr>
<th>Breathing volume</th>
<th>Body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>0.2 m\textsuperscript{3}/day</td>
</tr>
<tr>
<td>Human</td>
<td>20 m\textsuperscript{3}/day</td>
</tr>
</tbody>
</table>

For rats:

\[
\frac{5.3 \text{ mg/m}^3 \times 0.2 \text{ m}^3/\text{day}}{0.2 \text{ kg}} = 1.06 \text{ mg/kg/day}
\]

(3(a))

\[
\frac{1.06 \text{ mg/kg/day} \times 5 \text{ days}}{7 \text{ days}} = \text{Average exposure of 0.76mg/day}
\]

\[
\frac{1.06 \text{ mg/kg/day}}{0.2 \text{ kg}} = 5.3 \text{ mg/m}^3/\text{kg-day}
\]

For humans:

\[
\frac{5.3 \text{ mg/kg-day} \times 70 \text{ kg}}{371 \text{ mg/kg/day}} = 1.86 \text{ mg/m}^3/\text{day}
\]

(3(b))

\[
\frac{371 \text{ mg/kg/day} \times 20 \text{ m}^3/\text{day}}{1.3 \text{ mg/m}^3} = 29.7 \text{ mg/m}^3
\]

The fourth default approach for toxicity in tissues exposed via circulating blood following inhalation is based on the blood solubility of BE. Once the point of departure has been identified in the animal species, it is duration adjusted (as above), and the interspecies extrapolation is completed using blood:air partition coefficients (B:A PC). A dosimetric adjustment factor (DAF) is developed using the ratio of partition coefficients animal:human. The DAF differs from traditional uncertainty factors and CSAF values in that the point of departure is multiplied (rather than divided) by the DAF to complete species adjustment. The B:A PC for rats was set equal to the B:A PC for humans (7965) based upon experimental data indicating similar solubility of BE in rat and human blood (Corley \textit{et al.}, 1994). This yields a DAF of 1.

\[
5.3 \text{ mg/m}^3 \div 4 = 1.3 \text{ mg/m}^3
\]  

(4)

4.4.3. Comparison between PBPK adjusted and default approaches

For the interspecies extrapolation of the exposure concentration of BE, the use of the default approaches is highly uncertain. The uncertainty related to the “default” approach, in this case, arises from the conceptual model, parameters, and the toxic moiety, which are all essentially assumed to be “unknown” despite the availability of relevant chemical-specific TK data and models to inform these aspects. Default approaches yield human exposure concentrations ranging from 1.3 to 18.6 mg/m\textsuperscript{3}, while the application of the PBPK model for BE results in a human equivalent concentration of 10.6 mg/m\textsuperscript{3} (Fig. 10). The use of the PBPK model addresses a number of sources of TK uncertainty related to interspecies extrapolation including air phase mass-transfer characteristics, rates of metabolism, and production of toxic metabolites based on knowledge of mode of action, as well as other chemical-specific data. The PBPK model incorporates relevant data, then, to reduce the uncertainty in the risk assessment, by simulating AUC\textsubscript{BE-M} on the basis of physiological, biochemical, and physicochemical determinants in both test species and humans. Application of the PBPK model results in a value of 0.5 for AK\textsubscript{UF}, and a human equivalent concentration of 10.6 mg/m\textsuperscript{3}.

The application of the default approach to adjust for interspecies differences in toxicokinetics of BE is uncertain since the ‘dose metric’ (i.e., AUC\textsubscript{BE-M}) is not necessarily a simple function of body weight, body surface area, and the ventilation rate. Other species-specific factors that determine metabolism include breathing rate, fractional liver weight, liver blood flow and enzyme content/activity. Since the hematological effects are more closely related to the acetic acid metabolite BE-M in blood rather than the parent chemical concentration in the air or the amount inhaled, the use of BE-M as the dose measure (as simulated by the PBPK models) in the risk assessment is believed to be more certain than the default approach for interspecies extrapolation.

Given that the interspecies extrapolation focuses on the evaluation of the central tendency of the animal to human difference in kinetics, deterministic PBPK models are used for this purpose. Regarding the uncertainty in the “average” values of parameters of a PBPK model used for interspecies extrapolation, a relevant question is:

What is the impact of this parameter uncertainty on the simulations of dose metrics relative to the uncertainty associated
with the use of the available alternative approach (e.g., the default)?

The uncertainty related to the available alternative, i.e., default approach, arises from the conceptual model (based on nonspecific empirical observations), parameters (e.g., same for all chemicals and species; based on average body weight of 0.2 kg for the rat and 70 kg for humans) as well as the toxic moiety (i.e., unknown) (Fig. 11). PBPK models offer an opportunity to incorporate more data to inform the adequacy of or reduce the uncertainty associated with the default approaches, by simulating blood concentrations of the acid metabolite for BE on the basis of relevant physiological (breathing rate, volume of the target tissue, liver blood flow rate), biochemical (rate of metabolism), and physicochemical (partitioning of BE between blood and air, partitioning of BE between tissues and blood) determinants in both test species and humans. Despite the ability of the PBPK approach to more meaningfully address the TK uncertainty in the risk assessment, some uncertainty remains in that the relationship between BE exposure and acid metabolite BE-M formation was characterized at an exposure of 302 mg/m³ (6-h exposure), as compared to the point of departure exposure of 29.4 mg/m³ encountered 6 h/day, 5 days/week.

5. Discussion and conclusions

PBPK models provide a documentable and scientifically defensible means of bridging the gap between critical toxicity studies and human risk estimates by facilitating interspecies, interindividual, high dose to low dose and route-to-route extrapolations through incorporation of information on physiological scaling and chemical specific parameters. They shift the focus in risk assessments from external dose to internal dose, which is more closely associated with the tissue responses.

The complexity of PBPK models should be no more than is required for the risk assessment application for which they will be applied. The increased complexity and data demands of PBPK models must be counterbalanced by the need for increased accuracy, biological basis and scientific justifiability of any risk assessment application. While complex PBPK models may be relevant to chemicals for which the margin between exposure and effect is small, simpler models might be adequate for screening of large numbers of substances or preliminary assessments for individual chemicals to inform additional steps.

For PBPK models intended for application in risk assessment, the focus should be on purpose-specific “evaluation” rather than generic “validation”. In addition to comparing model predictions with PK data, critical analysis of aspects relating to the biological basis of the model structure and parameters as well as the reliability of dose metric predictions, supplemented with appropriate analyses of variability, uncertainty, and sensitivity is essential.

In order to facilitate transparency, reproducibility, and credibility, PBPK models need to be systematically characterized and documented for risk assessment application. The documentation should be sufficient to enable an experienced modeller, expert reviewer, or interested end user to evaluate a PBPK model and reproduce the input-output relationships for the dose metric of relevance to the risk assessment. Transparency could be improved through development of dedicated repositories for data, models, and their detailed documentation.

Communication between the modeller and the risk assessor is of critical importance in developing PBPK models applicable for risk assessment. The continuous involvement of a risk assessor right from the problem formulation stage would be key in informing the modeller to consider and address issues of relevance to MOA and risk assessment.

Mechanisms for adequate peer engagement for evaluating PBPK models in the context of their suitability for specific applications in risk assessment are essential. Enhanced access to modelling expertise through recruitment, training, or retraining, would facilitate greater uptake and optimal use of PBPK models by the risk assessment community.

Conflict of interest

The authors declare that there are no conflicts of interest.
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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jyrtph.2013.03.005.

References


