Generic physiologically based kinetic modelling for farm animals: Part II. Predicting tissue concentrations of chemicals in swine, cattle, and sheep


⁎ Corresponding author.
E-mail address: l.lautz@science.ru.nl (L.S. Lautz).

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ABSTRACT

The development of three generic multi-compartment physiologically based kinetic (PBK) models is described for farm animal species, i.e. cattle, sheep, and swine. The PBK models allow one to quantitatively link external dose and internal dose for risk assessment of chemicals relevant to food and feed safety. Model performance is illustrated by predicting tissue concentrations of melamine and oxytetracycline and validated through comparison with measured data. Overall, model predictions were reliable with 71% of predictions within a 3-fold of the measured data for all three species and only 6% of predictions were outside a 10-fold of the measured data. Predictions within a 3-fold change were best for cattle, followed by sheep, and swine (82%, 76%, and 63%). Global sensitivity analysis was performed to identify the most sensitive parameters in the PBK model. The sensitivity analysis showed that body weight and cardiac output were the most sensitive parameters. Since interspecies differences in metabolism impact on the fate of a wide range of chemicals, a key step forward is the introduction of species-specific information on transporters and metabolism including expression and activities.

1. Introduction

Animal health risk assessment of chemicals aims to protect a range of farm and companion animals from the harmful effects of chemicals present in the feed chain (Alexander et al., 2012; Toutain et al., 2010). It accounts also for impacts on human health due to transfer of chemicals into the food-chain (Alexander et al., 2012). For a given chemical, inter-species differences in toxicokinetics (TK) and toxicodynamics (TD) can be investigated through the quantification of inter-species variability in physiology, diet, absorption, metabolism, distribution and excretion (ADME), target receptors and toxicological sensitivity in different life-stages (Dorne and Fink-Gremmels, 2013).

Recently, the European Food Safety Authority (EFSA) recommended to further support quantitative risk assessment through a better understanding of such inter-species differences in TK and TD processes and the development of generic biologically-based models (EFSA, 2014). Such biologically-based models include tools for allometric scaling, physiologically based kinetic (PBK) and physiologically based TK-TD (PBTK-TD) models (Huang et al., 2015; Riviere et al., 2016). Generic PBK models integrate physiological and anatomical features subdivided into body compartments (i.e. organ volumes), connected through blood flow, chemical specific parameters (e.g. physico-chemical properties) and the compound’s biochemistry (e.g. Vmax and Km, metabolic clearance) (Brochot et al., 2007; Reddy et al., 2005). Model parameters correspond to physiological and biochemical entities specific to the body and compounds, such as affinities of the compounds for the tissues, or metabolic clearances. PBK models can be refined as chemical-specific, class-specific or generic for a given species or range of species and can be applied to a wide range of data poor compounds bearing in mind the underlying uncertainties associated with data gaps and poor characterisation of kinetics (Beaudouin et al., 2010; Clewell et al., 2004; Cohen Hubal et al., 2019; Edginton et al., 2006).

In the area of animal health, the use of kinetic information and PBK models is currently mostly limited to two main applications. The first one is on therapeutical dosing of veterinary drugs in a given species, the second one is on determining residue levels and transfer of regulated compounds (feed additives, pesticides, veterinary drugs) or contaminants (persistent organic pollutants, toxins) in animal products (e.g. meat, milk, eggs). Such carry over and residue levels can then be used as occurrence inputs and combined with human consumption patterns of animal products for human exposure assessment (Dorne and Fink-Gremmels, 2013; Huang et al., 2015; Riviere et al., 2016). A recent review assessed the available models in farm animal species and proposed the future development of generic PBK models in risk assessment.
(Lautz et al., 2019). In addition, generic models have been recently published for four fish species (fathead minnow, zebrafish, European stickleback, rainbow trout) (Grech et al., 2019). This manuscript describes the development and application of generic multi-compartment models in three farm animal species, namely swine, cattle, and sheep. Anatomical and physiological parameters were collected via meta-analysis and are presented in a related manuscript (Lautz et al., 2019). Model performance is illustrated in two case studies, i.e. one on melamine and one on oxytetracycline and a global sensitivity analysis was performed to identify the most sensitive model parameters.

2. Materials and methods

2.1. PBK model structure

The PBK model structure is generic for all three farm animal species and is presented in Fig. 1. Each model is structured with eleven compartments: arterial blood, venous blood, gastrointestinal tract (GIT), liver, heart, brain, adipose tissue, muscle, bone, lung, and kidney. Sheep and cattle have a twelfth compartment i.e. the milk compartment. All organs and tissues were modelled as homogenous compartments with a blood-flow limited distribution.

The model covers two exposure routes, i.e. oral exposure and intravenous injection. The GIT consists of two sub-compartments, the gut lumen and the gut tissue, enabling the inclusion of biliary excretion as a separate process. Absorption from the gut lumen into the gut tissue is modelled as a first order process and distribution is modelled throughout the body via the systemic circulation. Chemical elimination is included through different routes, i.e. renal excretion, hepatic metabolism, exhalation, egestion and accumulation in milk. The differential equations describing the mass balance in each compartment and parameters abbreviations are provided in the supporting information (SI, part 1).

2.2. Anatomical and physiological parameter values

An extensive literature search was performed to identify experimental data on anatomical and physiological parameters (e.g. organ volumes, relative blood flow). Meta-analyses were performed to define distributions reflecting intra-species variability in these parameters (Lautz et al., 2019). Data availability for blood flow parameters and their associated variability for the three species was limited. Data gaps were filled using allometric scaling (Lindstedt and Schaeffer, 2002) and a default variability of 30% was allocated as suggested by Clewell and Clewell (2008). Tissue composition parameters were expressed as fractions of neutral lipids, polar lipids, proteins and water. These fractions were reported for humans and assumed applicable to swine, cattle, and sheep (Schmitt, 2008).

2.3. Case studies

The PBK models were validated by comparing model predictions of tissue concentrations with experimental data measured in tissues of cattle, swine, and sheep for two compounds that are eliminated through renal excretion: melamine as an environmental contaminant and oxytetracycline as an antibiotic. An extensive search of the published literature in PubMed and Scopus using search terms 'species', 'substance' and 'tissue' yielded experimental data for these two compounds (see SI, part 3.1). Papers were included when the experiment was conducted in healthy animals, a clear description of the exposure was available, and measured tissue concentration was reported. Papers not fulfilling these criteria were excluded. The tissue:blood partition coefficients as well as the blood:air partition coefficient were calculated by available QSARs (Hendriks et al., 2005). The QSAR allowed the calculation of the chemical affinity for all tissues based on the octanol/water partition coefficient and tissue composition through considering the tissues constituents' lipids (both neutral and polar), proteins and water. For both compounds, characteristics, kinetic data and data for model evaluation are presented in Table 1. Model input parameters were predicted by allometric scaling or were based on values from peer-reviewed publications that were independent of the values used for model evaluation (see SI, part 3.2).

2.4. Global sensitivity analysis

Global sensitivity analysis of the model was performed for each species using the variance-based Sobol method, based on variance decomposition quantifying the relative contribution of each parameter to the total variance of the model output (Saltelli et al., 2008; Sobol’ et al., 2007). The global sensitivity analysis orders the model inputs by importance so that the main contributors to the variation in the output (e.g. blood concentration) can be identified. Sensitivity was assessed for oxytetracycline concentrations in the whole animal, blood, and kidney at three different time points, i.e. absorption phase, fast elimination phase, and slow elimination phase. Parameter values and exposure scenarios are described in detail in SI (part 2.1). Uniform distributions were used for all physiological parameters to compare model performance between species.

2.5. Software

The PBK models were implemented in the R software (version 3.3.3) (R Core Development Team, 2014). The function “soboljansen” in the “sensitivity” package was used to carry out the sensitivity analysis (Pujol et al., 2017). The R codes for the PBK models in the three farm animal species are presented in the SI (part 4) and are also available on the EFSA knowledge junction under the DOI [https://doi.org/10.5281/zenodo.3432796].
3. Results

3.1. Model evaluation and validation

For melamine and oxytetracycline, 19 publications were identified with experimental data and Figs. 2 and 3 illustrate the comparisons between their measured concentrations in different tissues and organs and the PBK model predictions. Tables S5, S6, and S7 summarise the fold-changes (FC) between the measured data and PBK model predictions for melamine and oxytetracycline (SI part 3.3). Globally, 71% of the model predictions were within a 3-FC of the measured data for all species and only 6% of the PBK predictions were outside a 10-FC. On a compound-specific basis, 40% and 79% of the predictions of the PBK models were within a 3-FC for melamine and oxytetracycline respectively and only 5–6% of the predictions were over- or under-predicted by more than a 10-fold. The predicted curves for blood concentrations were very close to the measured blood concentration-time data (Fig. 4). For other organs, single time point measurements were mostly reported from the literature. And at the tissue or organ level, 58–84% of the predictions in blood, kidney, and muscle were within 3-FC, whereas 67% of the predicted concentrations in liver were between 3-FC to 10-FC.

From an inter-species perspective, the model predictions showed relatively low variability with 82%, 76%, and 63% of predictions within a 3-FC for cattle, sheep, and swine respectively. Model predictions exceeded 10-FC in only 3% of cases in sheep and cattle and 9% in swine.

3.2. Global sensitivity analysis

Global sensitivity analysis of the PBK models for cattle, sheep, and swine with oxytetracycline shows that body weight (BW), cardiac output (CO) and renal blood flow (fCO_kidney) were the main contributors to the overall variance of the model output (Fig. 5). However, the relative contribution of each of those varied between cattle, sheep and swine. During the absorption phase, the model output for the blood concentration was impacted by the intestinal blood flow (fCO_intestine) as well as distribution of the chemical towards other organs such as muscle and adipose tissue (fBW_adipose, fCO_muscle). In the elimination phase, renal blood flow was the most sensitive parameter and had a strong influence on model outputs. The results of the global sensitivity analysis for model outputs with regards to whole animal and kidney concentrations were similar to that for blood concentrations (see SI part 2.2).

Table 1
Compound-specific parameters and data used for PBK model evaluation.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Parameter values and references</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class</td>
<td>–</td>
<td>Contaminant</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>g/mol</td>
<td>126.12</td>
</tr>
<tr>
<td>Log Kow</td>
<td>–</td>
<td>−1.37</td>
</tr>
<tr>
<td>Solubility</td>
<td>mg/L</td>
<td>3230</td>
</tr>
<tr>
<td>Vapour pressure</td>
<td>Pa</td>
<td>4.37E-08</td>
</tr>
<tr>
<td>Absorption rate constant (kabs)</td>
<td>min⁻¹</td>
<td>0.0059¹</td>
</tr>
<tr>
<td>Clearance (renal)</td>
<td>L/min/kg</td>
<td>0.001¹</td>
</tr>
<tr>
<td>References for model evaluation</td>
<td></td>
<td>(Battaglia et al., 2010; Cruywagen et al., 2011; Sun et al., 2011; Tkachenko et al., 2015)</td>
</tr>
</tbody>
</table>

* Poapolathep et al. (2015).
¹ Absorption rate constant for cattle and sheep (Schifferli et al., 1982).
² Absorption rate constant for swine (Pijpers et al., 1991).
³ Based on allometric scaling and dependent on the body weight of each species, equations are given in the SI (SI part 3.2).

Fig. 2. Comparison between concentrations measured in various organs of cattle (CA), sheep (SH), and swine (SW) and PBK model predictions for melamine. Dotted lines represent the 3-fold and 10-fold changes. Organs, species, and references of experimental datasets are indicated in legend: colours and shapes represent organs and studies, respectively.
4. Discussion

Generic PBK models have been developed for three farm animal species namely, swine, cattle and sheep. The performance of the models was illustrated and validated for melamine and oxytetracycline eliminated via renal excretion while comparing measured data and predicted outputs in these species. Overall, the generic models provided reliable predictions within 3-fold of the measured data.

For melamine, a data gap for melamine was identified with regards to absorption rate in the digestive tract of ruminants (Cruywagen et al., 2009, 2011). However, differences between exposure and excreted concentrations in ruminants suggest that the absorption of melamine is higher than 75%. In monogastric animals, such as swine, the absorption of melamine is nearly 100% and unchanged melamine is detected in urine only (Cruywagen et al., 2011; Dorne et al., 2013). Since absorption rates for melamine in the included species were not available in literature, melamine absorption rates were extrapolated from chicken, leading to uncertainty in the PBK model inputs for this parameter with still reliable predictions in cattle and sheep (Poapolathep et al., 2015). Overall, literature data on melamine in various tissues of the included species were very limited, so the quality of the included papers is of high relevance for the reliability of the model performance. Milk concentrations were overpredicted by the model in most cases when only about 2% of ingested melamine has been reported to be excreted in milk (Cruywagen et al., 2009). Nevertheless, melamine patterns in milk were dose dependent and variation was based on the milk yield which differed between studies (Battaglia et al., 2010; Cruywagen et al., 2009; Sun et al., 2011). This discrepancy might be due to the modelled blood flow for the mammary gland, which is higher compared to the renal blood flow. In swine, the organ concentrations in muscle and liver were overpredicted and showed large variability at high exposure concentration (1000 mg/kg). The rationale for such overpredictions are threefold. First, measured melamine tissue concentrations in swine showed large variability within the animals tested as well as between the measured concentrations of laboratories, leading to discrepancies between model prediction and observed data (Tkachenko et al., 2015). Second, experimental concentrations in swine were very high compared with those for sheep and cattle (Cruywagen et al., 2011; Sun et al., 2011; Tkachenko et al., 2015). However, the calculated clearance using allometric scaling was similar to measured clearance at high dose and steady-state conditions (0.072 vs 0.079 L/h/kg) (Wang et al., 2010). Third, due to limited data availability of experimental data for tissue: blood partition coefficients, tissue partitioning was estimated with a QSAR for polar chemicals (Hendriks et al., 2005) which is based on tissue constituents (e.g. polar lipids, non-polar lipids, water, protein) and octanol/water partition coefficient. This limitation however, did not affect the prediction of organ concentrations for sheep and cattle which were reliable.

For oxytetracycline, a veterinary antibiotic administered orally and intravenously, absorption is only partial in the swine’ intestine (Pijpers et al., 1991) and was not characterised in adult ruminants, i.e. cattle and sheep, and may vary compared to monogastric species. For other
substances, such differences in absorption between monogastric and ruminants already have been observed (Ratz et al., 1995). Oxytetracycline undergoes no metabolism and is excreted in urine unchanged (Nouws et al., 1985). Overall, measured blood concentrations were often available in literature, whereas tissue concentrations were scarce for more than one time point. For oxytetracycline, model predictions were reliable compared to observed data. However, Nielsen and Gyrd-Hansen (1996) reported very low blood concentrations after oral exposure, leading to overestimation of the model predictions, compared to similar studies in swine (Pijpers et al., 1990, 1991). Differences in the measured blood concentrations between the studies cannot be explained by small changes in the administered dose. Furthermore, kidney concentration of oxytetracycline in swine were underestimated by the model. These differences might be due to inclusion of the urinary formation in the kidney in the measured data, while the model predicts kidney tissue only (Black and Gentry, 1984). Another explanation may be the underestimation of tissue partitioning of oxytetracycline in the kidney. In the literature, a cattle and a sheep PBK model for oxytetracycline were developed to predict concentrations in different organs, after intramuscular administration and fitted model parameters based on experimental data (Achenbach, 2000; Craigmill, 2003). Based on this approach, the predicted concentration were within a 3-FC in 95% of cases (Achenbach, 2000; Craigmill, 2003). With regards to concentrations, our PBK model provided predictions with slightly lower accurate predicted values for cattle (82% predictions within a 3-FC) while for sheep, 76% of the predictions were within a 3-FC.

5. Conclusion and recommendations

Generic PBK models with species-specific physiological parameters were developed for three farm animal species. The models share the same structure for all three species with the exception of the milk compartment, which has been added for cattle and sheep as an extra elimination route of high relevance to food and feed safety. The model performance was illustrated for melamine and oxytetracycline which are renally excreted. In this context, further model validation is needed through PBK modelling of residues in edible tissues (e.g. muscle, kidney, liver) and animal-based products (i.e. milk) from cattle, swine and sheep for a range of compounds. However, a range of data gaps and recommendations for future work can be highlighted for these three species:

1. Peer-reviewed literature providing tissue residues and milk residues for a range of regulated compounds (pesticides, feed additives) and anthropogenic or naturally-occurring contaminants (e.g. persistent organic pollutants, mycotoxins) are still relatively scarce. Extensive literature searches and data collection from pre-market dossiers should be performed to develop kinetic databases to further explore the predictability of the models for a larger group of compounds.

2. Current generic model do not incorporate developmental aspects.
Further anatomical and physiological parameters should be collected to test the impact of growth and age on the PBK prediction and sensitivity.

3 Chemicals may undergo transport and phase I and/or Phase II metabolism and databases on species-specific information on transporters, enzyme expression and activities are currently not available. Enzyme expression and activities for these species may therefore be first collected from the literature or generated experimentally, when data gaps have been identified. Experimentally, a range of approaches can provide important datasets with regards to expression levels and activities including 1. OMICS technologies (i.e. transcriptomics, proteomics, metabolomics) providing qualitative and quantitative information on expression of enzymes and transporters. 1. Enzyme activities in liver microsomes and other organs (kidney, gut etc.) providing kinetic parameters for specific probe substrates (phase I, phase metabolism and transporters), regulated compounds and contaminants. In addition, integration of the in vitro metabolism, kinetic data and generic enzymes activities can also provide the basis to further develop quantitative in vitro to in vivo extrapolation models, which can be implemented in PBK models for animal risk assessment and ultimately limit in vivo testing in farm animal species.

Finally, these data gaps and recommendations can be broaden to a wider range of mammalian and avian species of public health and animal health importance. It is foreseen that these research efforts will lead to open source databases and PBK tools for multiple species and developmental stages and allow the implementation of a ‘One health’ approach in chemical risk assessment while integrating species differences in chemical kinetics and toxicity.

Declaration of Competing Interest

None.

Acknowledgment

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at https://doi.org/10.1016/j.toxlet.2019.10.008.

References


