Extending the 3R Principle Using PBPK Modelling: A Case Study of TCDD Transfer from Feed to Growing Pigs

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Introduction

2,3,7,8-dibenzo-p-dioxin (TCDD) is a ubiquitous contaminant and unwanted byproduct of industrial and combustion processes. It is known for its extraordinary toxicity and bioaccumulative properties[1]. The main source of TCDD exposure for humans is consumption of contaminated food, mainly meat, fish and dairy products[2].

A general approach to quantify the amount of toxins transferred from feed to food is to establish a physiologically based pharmacokinetic (PBPK) model. In the context of health risk assessment a reliable PBPK model serves the following aims: 1) to estimate the maximum amount of contaminants in the feed, so that their final concentration in the animal tissues is within the allowed range; 2) to evaluate the time of substantial substance elimination from an animal, while it is fed with non-contaminated feed; 3) to predict the main sources of undesirable exposure[3].

There are several PBPK models describing TCDD absorption, distribution, metabolism and elimination (ADME) in various species[4-8]. However, a PBPK model for the transfer of TCDD from contaminated feed into pigs is missing. TCDD rarely contaminates the food chain as an isolated compound and is instead accompanied by a wide range of PCDD/F congeners[9]. Yet, since the pharmacokinetic properties of individual molecules vary dramatically, it is reasonable to study dioxin-like compounds individually. Thus, establishing of a PBPK model for TCDD in pigs will be the first step to establishing general non-invasive animal experiments of the transfer of chemicals.

PBPK model parametrization requires the information on the toxin concentrations in body fluids and tissues of target organs[10], which are measured either after biopsy sampling or slaughter of an animal. But, according to 3R principle to animal experiments (Replace, Reduce, Refine), a more humane way of conducting a research would be to avoid invasive measurements and leave only sampling of blood, urine and feces[11]. Missing data on substance distribution in the body could be modelled using the partition coefficients of the chemical within the tissues.

Development of a proper theoretical basis for the calculation of equilibrium partition coefficients has become a separate research field. The most accurate approach to estimate these values is to use polyparameter linear free energy relationships (PP-LFERs)[12]. All published information that is necessary for *in silico* calculations has been collected in an on-line database. The approach can be used for estimation of partitioning between heterogeneous biological systems, such as blood and tissues[13].

A practical application of these *in silico* predicted partition coefficients we pursue here is to refine invasive animal experiments yielding the data for PBPK parametrization in the spirit of the 3R principle. The goal of this research is to test the applicability of the data from the Goss et al. database for establishing a PBPK model of TCDD transfer from feed into growing pigs.

Materials and methods

Data for model parametrization.

A three-compartment PBPK model for TCDD ADME in growing pigs was established (Fig. 1). It is based on the work of Wang et al[8]. The physiological values were taken from various published sources[14-19, 8]. When the information for pigs was not available, data on other animal species, namely, rat and human, were used as initial estimates. The growth of the animals is approximated with logistic equation.

Shen et al.'s experimental studies on TCDD pharmacokinetics in pigs were used for model parametrization[20, 21]. During the research the piglets were divided into 3 experimental groups. Each group was consuming feed contaminated with a corresponding amount of TCDD. Its concentrations in adipose tissue and liver were measured. This research provides us with the most detailed data on TCDD kinetics in pigs published so far.

Pharmacokinetics and pharmacodynamics of TCDD.

In living organisms dioxins are metabolized by enzymes of the cytochrome P450 family. Their metabolic activity is species-dependent[22]. In the case of pigs, TCDD can bind specifically to porcine CYP1A1, but, in contrast to rats and dogs, there is no explicit evidence of TCDD metabolism by swine hepatocytes published so far[23]. Therefore, TCDD metabolism step in the PBPK model for pigs can be considered as interspecies interpolation.

Another disputed aspect of TCDD pharmacokinetics is its dose-dependent absorption. It was noticed that the fraction of TCDD absorption in rats increases as the dose decreases[8], while some studies claim that the absorption is a constant parameter[24].

Due to the lack of information about TCDD absorption and metabolism in pigs, two types of PBPK model intending to describe ADME of TCDD were established and compared: the first model considers dose-dependent TCDD absorption in gastrointestinal tract and ignores inducible TCDD metabolism in liver, while the second model considers self-inducible metabolism and dose-independent absorption.

Model with dose-dependent absorption.

Each of the three experimental groups has its own adjustable parameter for the fraction of absorbed TCDD, which is fitted individually in each case. TCDD elimination is described as clearance from the blood compartment. The mathematical description of the model is based on the mass balance in each compartment.

Model with self-inducible TCDD metabolism.

Although TCDD is reported to be a highly persistent and bioaccumulative substance, there is evidence of its self-inducible metabolism in rats' liver, which occurs according to the following the scheme: TCDD diffuses through the cell membrane into the hepatocyte and binds specifically to the aryl hydrocarbon receptor (AhR) forming a complex with some other proteins. After further transformations the complex is transferred into the nucleus, where it binds to dioxin responsive elements (DRE) on DNA. As a result, transcription and translation of CYP1A2 are induced. As a consequence, the rate of TCDD metabolism rises proportionally to the increase of CYP1A2 concentration in the cell[15, 16]. Equations 1 – 3 describe the pharmacodynamic processes occurring with TCDD in hepatocytes[25, 6, 8]. Mass balance equations remain the same as for the model with dose-dependent absorption, apart from the metabolic clearance of TCDD in the liver.

$$Stimulus = 1 + InA2 \cdot \frac{{C_{TCDD-AhR}}^h}{{ICA2}^h + {C_{TCDD-AhR}}^h} \quad \text{(Eq. 1)}^{25};$$

$$\frac{d}{dx}\left(C_{CYP_{induced}}\right) = k_{in} \cdot Stimulus - k_{out} \cdot C_{CYP_{induced}} \quad \text{(Eq. 2)}^{25};$$

$$K_{metabolic} = \frac{C_{CYP_{induced}} - C_{CYP_{basal}}}{C_{CYP_{basal}}} \cdot K_{pig} \quad \text{(Eq. 3)}^{6};$$

Using *in silico* predicted partition coefficients

Comparison of *in silico* partition coefficients with experimental data is possible only, when the substance of interest is equilibrated among the considered media. For a living organism, it may be assumed that a toxin is in pseudo-equilibrium, when its elimination rate is low enough and enough time has passed. Given that TCDD is a poorly metabolized substance, it seems to be a promising candidate for a chemical that is quasi-equilibrated within compartments.

If it appears that TCDD is not in a quasi-equilibrium state, there is still a possibility to check the capability of the Goss' database[13] to estimate partition coefficients in biological systems. For this purpose, a PBPK model should be established first. Afterwards, the model should be modified, so that the toxin clearance rate is set to zero, and all TCDD stays in the organism. The resulting concentrations of the substance in compartments should be used for validation of the partition coefficient value. For that case, unfortunately, the Goss' values would not allow the substitution of animal experiments, even if the *in silico* partition coefficients coincide with the modelled results.

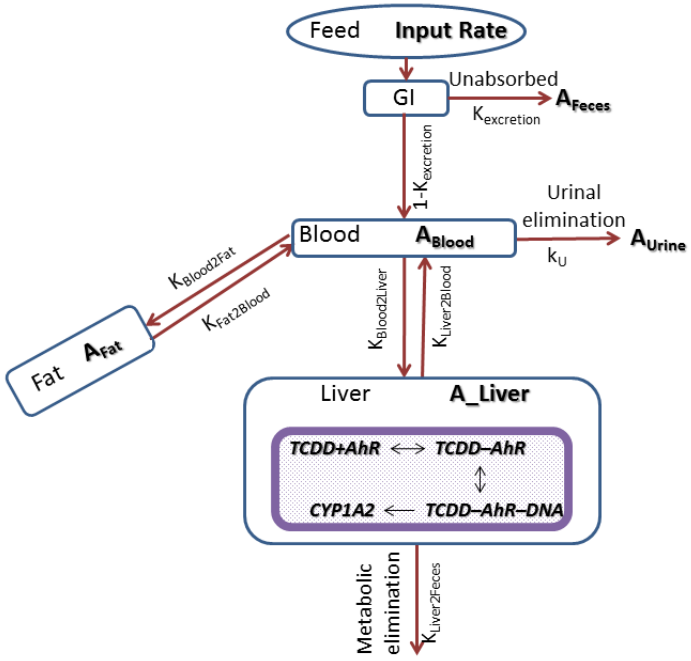

Figure 1. *Scheme of a 3-compartment PBPK model of TCDD transfer from contaminated feed into pigs.*
1. The basic model has a constant value for the excreted fraction of TCDD from gastrointestinal tract and does not consider TCDD metabolism in liver (shown in violet frame).
2. The model with dose-dependent absorption has different values of $K_{excretion}$ for each experimental group.
3. The model with self-inducible TCDD metabolism has constant value of excreted fraction of TCDD from gastrointestinal tract and considers molecular processes in liver (shown in violet frame).

Results and discussion

The basic model without additional assumptions about absorption or metabolism could not be parameterized properly, while the two models, which consider either dose-dependent absorption, or self-inducible metabolism, can describe the experimental data. However, based on the experimental data available, it is not possible to decide, which of the assumptions is more realistic. Consequently, further studies of TCDD pharmacokinetics are necessary. In this sense, humane and refined experiments, which include the measurements of TCDD amount in feces, but not removal of tissues, would be enough to prove or reject the hypothesis of dose-dependent absorption of TCDD in pigs. Data on TCDD concentration in porcine blood and urine is also not published, although these values are of crucial importance for reliable parametrization of a PBPK model and so those experimental results would be helpful.

The experimental measurement of TCDD concentration ratio between adipose tissue and liver is not in line with the value of partition coefficient predicted by the database. Moreover, partition coefficients predicted by both models do not coincide with the *in silico* values. However, the direct estimation of partition coefficients is based among other things on the general tissue composition and does not take into account specific binding of a toxin to proteins. Thus, TCDD can bind with high affinity to AhR, CYP1A2 and, additionally, to CYP1A1[15, 16, 23, 22].

Moreover, the basal level of CYP1A2 in hepatocytes is a dynamic value and depends on the age of the animal[26], but the dynamics of porcine CYP1A2 expression have not been studied yet. Therefore, the values of CYP1A2 concentration in liver cells cannot be extrapolated to the whole time span of an experiment. So, one of the further steps to test the applicability of Goss' database for prediction of TCDD partition within biological tissues would be to exclude from the calculation the fraction of TCDD molecules that are bound to the mentioned proteins.
In summary, the presented work serves as the basis for further development of PBPK model of TCDD transfer from contaminated feed into growing pigs, which will be revised and validated after additional measurements are performed. Deeper insight into chemical interactions of TCDD with liver proteins would allow us to conclude whether it is possible in the case of TCDD to substitute invasive measurements on experimental animals with *in silico* predicted partition coefficients.

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