RISK III

Mathematical modeling of Ah-receptor regulated P450 gene expression and its application in the risk assessment of 2,3,7,8-TCDD

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1. Introduction

The toxic potency of individual dioxins is expressed by their Toxicity Equivalency Factor (TEF). In attributing TEFs to dioxins several toxic endpoints are taken into consideration. Though being a useful method this way of scaling dioxin toxicity is complex: different endpoints with (assumed) different working mechanisms have to be scaled towards each other. In practice this approach therefore only allows a semi-quantitative determination of TEFs. A pure quantitative way of attributing TEFs is to strive for a more uniform way of scaling dioxin toxicity. This goal may be achieved by classifying toxicity according to common toxic working mechanisms, quantifying this mechanism (or its main determinant) and using the quantified mechanism as the starting point for the calculation of TEFs. In this paper we illustrate this principle for the calculation of TEFs for Ah-receptor related toxicity.

In mammals the Ah-receptor is an important regulatory protein for cellular differentiation and biochemical cell functioning. In the growing organism the Ah-receptor is involved in the regulation of organ development[1)] whereas, in the adult organism, it regulates the induction of P450 metabolism[2)]. As a consequence the exposure to Ah-receptor ligands such as dioxins is expected to lead to a widely different spectrum of toxic responses. For example, dioxins may interfere with the physiological function of endogenous Ah-receptor ligands, a process expected to lead to the disturbance of organ development[1)]. Furthermore, the induction of P450 proteins may lead to disturbed metabolism of endogenous P450 substrates such as estrogens. Disturbed estrogen metabolism is believed to be related to dioxin induced hepatic carcinogenesis[3)]. Also dioxins may specifically accumulate in the organs as a consequence of the induction of P450 proteins[4)]. Quantifying Ah-receptor interactions thus allows the scaling of a chemical's bioaccumulating potency and its potency to disturb cellular development and cellular metabolism.

Notwithstanding the importance of the Ah-receptor as a mediator of dioxin toxicity dioxin-Ah-receptor interactions have at the regulatory level only been marginally taken into consideration. The bioaccumulating potential of 2,3,7,8-TetraChloroDibenzoDioxin (TCDD) has indeed been used as the starting point for the calculation of the TDI for this compound[5)]. However, this calculation was based on *ad hoc* assumptions of the accumulation of TCDD in the organism and did not specifically incorporate the mechanism which is held responsible for this phenomenon, i.e. the induction of Ah-receptor dependent P450 proteins.

Physiologically Based PharmacoKinetic (PBPK) modeling of ligand-Ah-receptor interactions offers a good opportunity for the development of Ah-receptor based TEFs. Recently, a few PBPK models incorporating Ah-receptor dependent P450 induction by TCDD have been proposed[6),7)]. These models however, defined Ah-receptor dependent P450 induction, though being a cellular process, as a mechanism essentially taking place at the organ level. Also in these models the problem of the identifiability of model parameters was not taken into consideration. This restricts the application of these models in calculating Ah-receptor based TEFs. To overcome this we developed a model for Ah-receptor dependent P450 induction at the cellular level and incorporated this model into the liver compartment of a PBPK model for TCDD. We defined this model in such a way that it is suited for the calculation of Ah-receptor based TEFs. In the calibration of the model we especially paid attention to the problem of the identifiability of its parameters.

2. Model definition

In short the model was defined as follows. For a particular gene P450 induction was modeled as a sequence of events starting with the binding of TCDD to the Ah-receptor and the binding of the TCDD-Ah-receptor complex to gene specific DNA sequences (Dioxin Responsive Elements) and ending with P450 mRNA and P450 protein synthesis. The potency (TEF) of TCDD (and any other dioxin) to affect the functioning of a specific Ah-receptor regulated protein was expressed by the model parameter μ. This parameter characterizes the efficiency of the binding of a ligand to the Ah-receptor and of the binding of ligand-Ah-receptor complex to DNA. Mathematically μ is defned as:

$$\mu = \frac{K_{d,L\text{-}AhR} \; K_{d,L\text{-}AhR\text{-}DRE}}{A_0 + K_{d,L\text{-}AhR\text{-}DRE}}$$

with:

$K_{d,L\text{-}AhR}$	Dissociation constant for the binding of the ligand (L) to the Ah-receptor (AhR)
$K_{d,L\text{-}AhR\text{-}DRE}$	Dissociation constant for the binding of the ligand-Ah-receptor complex (L-AhR) to gene specific Dioxin Responsive Elements
A_0	Cellular Ah-receptor concentration

The way μ is defined makes its calibration on the basis of Ah-regulated gene expression data (mRNA and/or protein synthesis) possible. In contrast with the other models[3),6)] we assumed the elimination of TCDD to depend on Ah-receptor dependent P450 induction. The cellular P450 induction model was incorporated into the liver compartment of a PBPK model for TCDD. The PBPK model consisted of five organ compartments (blood, liver, fat, richly perfused organs and slowly perfused organs). With the exception of the fat compartment[7)] the PBPK model was perfusion limited.

3. Model calibration

The model described above contains 30 model parameters. Except ten, all parameters were obtained from the literature. The others were obtained by model calibration, i.e. by fitting the model to a specific experimental data set. For the calibration of the model we selected an acute[8] and a chronic[9] study on P450 induction by TCDD in the rodent liver. The acute study consisted of a time-course study on the disposition of a single dose of TCDD in the liver and the fat tissue and of a disposition study of TCDD of several doses. The model was calibrated to the time-course study. The calibrated model was used for the simulation of the kinetics of TCDD in the dose-disposition study. Without changing its parameter values the model described the latter study quite well. The chronic study selected for the calibration procedure consisted of a repeated administration of TCDD at four dose levels. When the model calibrated on the acute exposure to TCDD was used for the simulation of the chronic study it systematically overestimated the hepatic disposition of TCDD. Slightly recalibrating the model parameter characterizing the elimination of TCDD from the body however led to a model that, for all four dose levels tested, gave a satisfactory description of the hepatic disposition of TCDD after chronic exposure.
Given the available experimental data sets the model parameters characterizing the potency of TCDD to induce hepatic Ah-receptor dependent P450 synthesis could be identified ($K_{d,L-AhR}$ = 28 pM; $K_{d,L-AhR-DRE}$ = 0.2 nM; A_0 = 1.1 nM). The TEF of TCDD for Ah-receptor regulated P450 induction (μ) was determined at 4 pM.
The use of *in vitro* tissue culture techniques for the estimation of Ah-receptor based TEFs will be discussed. Furthermore model simulations of a PBPK model incorporating Ah-receptor interactions of mixtures of dioxins will be presented. Finally the validity of using toxicokinetic modeling of dioxins, especially TCDD, as the basis for the calculation of TDIs will be reviewed.

In summary we developed a PBPK model incorporating Ah-receptor dependent P450 induction for single compounds and mixtures of dioxins. The model was 1) used for the calculation of Ah-receptor dependent TEFs for dioxins and 2) used to improve toxicokinetic modeling as the starting point for the calculation of the TDI of dioxins.

4. References

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