Physiologically-Based Pharmacokinetic Model for TCDD in C57BL/6J Mice

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INTRODUCTION

Physiologically-based pharmacokinetic (PBPK) models can be used as tools for the integration of absorption, distribution, and disposition kinetics of a chemical. They also offer the advantage of permitting extrapolation between species, assisting in understanding important factors that influence the physiologic response to a certain chemical and directing further research. In this project, a previously developed PBPK model for TCDD in rats (Andersen et al.)[1], which incorporates diffusion-limited uptake and nonlinear disposition in tissues is extended to describe the pharmacokinetics and pharmacodynamics of TCDD in C57BL/6J mice.
The objectives of the present work were to extend the model to mice and test the model against experimental data describing (i) CYP1A1 and CYP1A2 induction measured by enzyme activity and protein changes and (ii) TCDD elimination rate changes as a function of exposure concentrations in C57BL/6J mice.

METHODS

Model Structure The model consists of a blood compartment and six tissue compartments: liver, fat, muscle, skin, kidney, remaining slowly perfused tissues and richly perfused tissues. Each compartment in the model has an associated partition and capillary permeation coefficient representing intrinsic tissue solubility and diffusion-limited uptake from tissue blood, respectively.
The liver compartment is modelled with equations to describe free dioxin, first-order rate of metabolism, and saturable binding of TCDD to both the *Ah* receptor protein and to an inducible binding species thought to be CYP1A2 protein. Induction of both CYP1A1 and CYP1A2 were described as an increase in synthesis rate specified as a Hill-type relationship between the TCDD-*Ah* receptor complex and DNA binding species. Oral absorption is described as the first-order uptake from the gastrointestinal tract into liver blood.

Model parameters Physiological parameters were obtained from the literature. Initial estimates of partition coefficients for TCDD were from literature (Leung et. al ,1990[4]) and were adjusted to fit the observed tissue concentrations. Rate of metabolism was estimated from fits to total urinary and fecal excretion data.
Protein levels of CYP1A1 and CYP1A2 measured by Western Blots and normalized to a standard hepatic sample were used to obtain values for maximal-fold induction of both the proteins. The synthesis and degradation rates, and the Hill term were estimated from fits to CYP1A1 and CYP1A2 protein data by keeping *Ah* receptor parameters fixed. Ratio of rate of synthesis to degradation defines the basal level of the two proteins and correspond to experimental measures. Binding constant for TCDD binding to the Ah receptor and CYP1A2 proteins were obtained from literature [Okey et al., 1989[6] and Poland et al., 1989[7], respectively]. Levels of the receptor protein were used as obtained by Okey et al., 1989[6].
The same model parameters were used for all the data analyses. The mass-balance differential equations for the model, were solved simultaneously using Simusolv (Dow Chemical Company, Midland, MI).

Data for analysis All studies were conducted in male C57BL/6J mice which were 4-months old at the time of initiation of te exposure and weighed 28-32g.
Treatment. Mice were given a single oral dose of 0, 0.05, 0.25, 0.5, 2.5, 5, 25, or 50 ug [^{3}H]TCDD/kg body wt. (specific activity of 75 uCi/ug TCDD) in a corn oil vehicle and held for 1,4 and 28 days. The dosing volume was 10 ml/kg body wt. Urine and feces were collected daily.
Sample analysis. Radioactivity in tissues was determined by combustion (packard 306B Biological Oxidizer, Downers Grove, IL) to ^3H_2O, followed by liquid scintillation spectrometry.
Microsomal fractions were prepared from liver tissue and analyzed for ethoxyresorufin O-deethylase (EROD) activity, a marker for CYP1A1 and acetanilide-4-hydroxylase (ACOH), a marker for CYP1A2 activity. Microsomal protein was immunoblotted to quantitate CYP1A1 and CYP1A2 protein.

The model is used to describe time-course (1,4 and 28 days) and dose-response (0.05, 2.5 and 50 ug/kg oral exposure) data in C57Bl/6J mice for tissue dosimetry (liver, fat, muscle, skin and kidney), excretion over 28 days, and induction of CYP1A1 and CYP1A2 enzymes measured using enzyme activity and protein changes.

RESULTS AND DISCUSSION

The KA value (absorption rate constant) estimated to describe the uptake of TCDD was 0.05 hr^{-1}. Model predictions for tissue dosimetry at day 1 and 4 are in good agreement with the observed data. The time course behavior for the liver and fat concentrations was sensitive to the diffusion coefficients, fat partitioning, and binding affinities of the liver protein. Model simulations and data required to introduce a rapid uptake in the fat compartment to describe day 1 concentration profiles as compared to the liver, kidney and richly perfused compartments, but with a lower partition coefficient than described in literature (PF = 100 as compared to 300 used by Leung *et al.*[3]). The concentration profiles on day 4 and day 28 were not sensitive to these parameters. The liver and fat concentrations at day 28 were more sensitive to the rate of metabolism. Concentrations in kidney, muscle and skin were appropriately described by the model on days 1 and 4. There was about a 2-fold over-prediction in these tissues, and the liver and fat on day 28 at the medium and high dose. This consistent deviation of predictive values from experimental results need to be further evaluated. The very high rate of blood flow to the kidney, lipid content of the skin and the very large pool of the muscle are factors that may influence the distribution to these tissues and are factors that need further characterization.
Accumulation in the liver is thought to be due to the induction of the specific binding protein, CYP1A2. The hepatic concentrations at the low dose was sensitive to the binding affinity. The binding constant for CYP1A2 set at 30 nM gave the best description. This does not vary considerably from the in vitro measurements of 56 ± 16 nM measured by Poland *et al.*[7] (1989).
The total excretion of TCDD metabolites in urine and feces was seen to be most affected by the rate of metabolism. A good description of the excretion at 0.05 ug/kg was obtained with a single first-order rate of metabolism. To describe excretion at 2.5 and 50 ug/kg doses it was necessary to increase the metabolic clearance by incorporating a proportionality constant to the first-order rate. This constant is related to increases in CYP1A2 protein and was set at 0.06 resulting in a maximum increase of ~2.5-fold.
The induction of metabolic elimination was included in the model based on half-life measurements in the liver and fat tissues determined from concentrations measured in these tissues. TCDD half-life decreased with an increase in the tissue concentration in both fat and liver, changing from 16.5 and 28.9 days in the liver and fat, respectively at 0.05 ug/kg to 11.6 and 12.9 days at 50 ug/kg. TCDD has not been shown to induce its own metabolism in either rats or mice. In vitro studies have also been unable to show any induction of metabolism. But the model does not explain the excretion profiles with a single first order rate of metabolism, and the proportionality constant which includes a maximum 2.40-fold increase in metabolism is required to

explain the excretory profiles. The fold-increase for metabolic clearance predicted from simulations in this study correlate with observations of Leung *et al.*[5] (2.4 and ~ 2), although that study was conducted at 10 ug/kg with ip dosing.
It was necessary to include an approximately 40-fold induction of CYP1A2 protein from the basal levels, to describe the tissue dosimetry. This elevated fold induction was not observed with enzyme activity (Acetanilide hydroxylation) measurements (only ~ 7-fold induction) and did not describe the tissue dosimetry. Literature experiments provide ample evidence that acetanilide is not a specific substrate for CYP1A2 (Lang et al. 1981[2]) leading to the hypothesis that the basal level of activity measured in control animals was the total of catalytic activity for hydroxylation by different isozymes, but the measurements in induced animals was significantly due to CYP1A2. To correlate the protein and activity measurements a percentage (~75%) of basal activity was attributed to enzymes other than CYP1A2. This was introduced as a constant factor in an activity equation related to protein (induction described above) at any given time. The activity equation and the model was used to simulate profiles for CYP1A2 induction measured by activity at all days. This approach provides a good correlation for both activity and protein.
Unlike CYP1A2, CYP1A1 activity provides a good correlation with protein ($r = 0.76$), and the model provide good predictions for the activity measurements based on the protein data on all doses and days except 50ug/kg dose on day 1. This is a first attempt to incorporate both protein and activity measurements in the model and may require further refinement.
This model provides a good description of both pharmacokinetic and pharmacodynamic measurements (enzyme induction). This model is a refinement over previous mouse models for TCDD in that diffusion-limited uptake is incorporated, which is the behavior of TCDD and related chemicals. The model also incorporates parameters for hepatic binding species from experimental observations in terms of CYP1A2 induction and correlates liver sequesteration in a dose and time-dependent manner. A realistic biologically based model for TCDD would be one that is validated with studies in different species, doses and routes of exposure, and extending to humans. The extension of the rat model to mice supports the robustness of this PBPK model to accurately predict the disposition of dioxin in various rodent species.

(This does not necessarily reflect EPA policy)

References

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