Extrapolation of a PBPK Model for Dioxins across Dosage Regimen, Gender, Strain, and Species

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A physiologically based pharmacodynamic (PBPK) model for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) was developed based on pharmacokinetic data from acute oral exposures of TCDD to female Sprague-Dawley rats (Wang et al., 1997, Toxicol Appl Pharmacol 147, 151–168). In the present study, the utility of this model to predict the disposition of TCDD in male and female Sprague-Dawley and female Wistar rats exposed to TCDD through different dosage regimens was examined. The ability of the model to predict the disposition of 2-iodo-3,7,8-trichlorodibenzo-p-dioxin (ITrCDD) in mice (Leung et al., 1990, Toxicol Appl Pharmacol 103, 399–410) was also examined. The ability of the model to predict across routes of exposure was assessed with intravenous injection data (5.6 μg/kg bw) (Li et al., 1995, Fundam Appl Toxicol 27, 70–76) in female rats. Analysis across gender extrapolations used data for male Sprague-Dawley rats exposed intravenously to 9.25 μg TCDD/kg bw (Weber et al., 1993, Fundam Appl Toxicol 21, 523–534). The analysis of across-dosage regimen and stains of rats extrapolations were assessed using data from rats exposed to TCDD through a loading/maintenance dosage regimen (Krowke et al., 1989, Arch Toxicol 63, 356–360). The physiological differences between gender, strain, and species were taken into account when fitting the PBPK model to these data sets. The results demonstrate that the PBPK model for TCDD developed for female Sprague-Dawley rats exposed by acute oral dosing accurately predicts the disposition of TCDD, for different gender and strain of rats across varying dosage regimens, as well as in a strain of mice. Minimal changes in fitted parameters were required to provide accurate predictions of these data sets. This study provides further confirmation of the potential use of physiological modeling in understanding pharmacokinetics and pharmacodynamics.

Key Words: 2,3,7,8-tetrachlorodibenzo-p-dioxin; physiologically based pharmacokinetic modeling; pharmacokinetics; species extrapolation; risk assessment.

The quality and quantity of dose response and pharmacokinetic data for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) has aided in the development of physiologically based pharmacokinetic (PBPK) and biologically based pharmacodynamic (BBPD) models in rats and mice (Andersen et al., 1991; Andersen et al., 1993; Buckley, 1995; Kedders et al., 1993; Kohn et al., 1993; Leung et al., 1998; Santostefano et al., 1998; Wang et al., 1997). The overall goal of these modeling efforts is the development of PBPK and BBPD models that can provide accurate predictions of tissue concentrations of environmental contaminants in exposed populations. The published PBPK models have undergone a number of iterations between development, validation, and refinement. However, the BBPD efforts are in the early stages of development. While validation of these models is inherently difficult, their utility can be examined by comparing the ability of the model to predict multiple data sets that employ different dosage regimens as well as different gender, strain, and species of laboratory animals. Models that can predict multiple experimental data sets using different species and different dosage regimens may also provide accurate predictions of tissue concentrations from a variety of human-exposure scenarios.

There are a number of consistent findings on the toxicity and pharmacokinetics of TCDD across species, and this consistency increases the confidence that PBPK models may be used in species extrapolations. For example, the biological and toxicological effects of TCDD are mediated through the Ah receptor (Birnbaum, 1994; Gasiewicz, 1997; Hahn, 1998; Rowlands and Gustafsson, 1997; Wilson and Safe, 1998), which is a ligand-activated transcription factor present in all mammalian species examined (Hahn, 1998). TCDD has a long half-life in all species examined ranging from weeks to years.
(Van den Berg et al., 1994). In a number of mammalian species there is a dose-dependent hepatic sequestration of TCDD (Van den Berg et al., 1994; DeVito et al., 1998; Diliberto et al., 1995,1997, 1999; Abraham et al., 1988). Initial studies indicated that the hepatic sequestration was an Ah receptor (AhR) mediated response (Poland et al., 1989a,b). Recent studies demonstrate that CYP1A2 is the inducible TCDD-binding protein responsible for the hepatic sequestration (Diliberto et al., 1997, 1999). The mammalian PBPK-BBPD models developed for TCDD include an AhR-mediated induction of an hepatic TCDD-binding protein to account for the hepatic sequestration (Andersen and Greenlee, 1991; Andersen et al., 1993; Buckley, 1995; Kedderis et al., 1993; Kohn et al., 1993, 1996; Leung et al., 1990a,b; Wang et al., 1997; Santostefano et al., 1998).

While there are similarities in the pharmacokinetics of TCDD between species, there are also subtle differences between species that must be incorporated into PBPK models. For example, in rats, TCDD and its metabolites are predominantly eliminated in the feces (Abraham et al., 1988, van den Berg et al., 1994). In the mouse, urinary elimination of TCDD metabolites accounts for a greater extent of elimination than in rats; however, for both species, urinary elimination is a minor route (Birnbaum, 1986; Gasiwicz et al., 1983). Fortunately the TCDD metabolites are essentially non-toxic and species differences in the route of elimination of these metabolites would contribute little to the differences in the toxicity of TCDD. There is suggestive evidence that high doses of TCDD increases its own metabolism in dogs (Poiger and Schlatter, 1985). In rats, there is little evidence that TCDD induces its own metabolism. While in vitro studies of rat hepatocytes suggest that high doses of TCDD could increase its metabolism (Olson et al., 1994; Tai et al., 1993), in vivo studies in rats demonstrate little evidence of autoinduction of TCDD metabolism (Kedderis et al., 1991).

The pharmacokinetics of TCDD and related chemicals are controlled primarily by several factors: membrane permeability; Ah-receptor concentrations; basal and inducible CYP1A2 concentrations; and binding affinities to the Ah receptor and CYP1A2 (Santostefano et al., 1998; Wang et al., 1997). The PBPK-BBPD models developed for TCDD should accurately predict across gender and strain of rat as well as across dosage regimens for several reasons. First, membrane permeability is determined by the structure and physical chemical properties of the membrane as well as solutions exposed to the membrane. These parameters should remain fairly constant across strains of rats, although the dosing vehicle and route of exposure can influence estimates of the membrane permeability. Second, the basal CYP1A2 concentration and the AhR concentration in different tissues are also expected to be similar in both male and female animals. Previous studies also demonstrate that the difference in CYP1A2 induction between male and female rats is minimal and decreases with increasing dose (DeVito et al., 1996). The binding affinity to the Ah receptor does not appear to vary dramatically between strains of rats (Pohjanvirta and Tuomisto, 1994). In contrast, the binding affinity of TCDD to the AhR does vary significantly between mouse strains (Poland et al., 1994). There is suggestive evidence that the binding affinity of TCDD to the human Ah receptor also varies by approximately a factor of 10 (Micka et al., 1997).

If the structure of the model is appropriate, then the model developed in one species or strain should describe the pharmacokinetics in another species or strain if species-specific or strain-specific parameters are modified such as body and organ weights, blood flows, Ah receptor and CYP1A2 concentrations and binding affinities. Previously, this laboratory developed a PBPK-BBPD model to describe the time- and dose-dependent tissue distribution of TCDD and induction of CYP1A1 and CYP1A2 in multiple tissues after a single acute oral exposure to TCDD in female Sprague-Dawley rats (Santostefano et al., 1998; Wang et al., 1997). This manuscript examines the utility of this PBPK-BBPD model by testing its ability to predict tissue concentration across different routes of exposure (po, iv, and ip), dosage regimens (acute vs. subchronic), gender and strain of rats, species (rats and mice), and chemicals (TCDD vs. \([{}^{125}\text{I}]\)-2-iodo-3,7,8-trichlorodibenzo-p-dioxin [ITrCDD]). The data used in this exercise are from previously published data sets from this and other laboratories (Krowke et al., 1989; Leung et al., 1990b; Li et al., 1995; Wang et al., 1997; Weber et al., 1993).

### MATERIALS AND METHODS

The model examined in the present study has been described in greater detail in two previous publications (Santostefano et al., 1998; Wang et al., 1997). This model was fit to several published data sets from other laboratories and the approaches used to conduct the examination of the model are described below. Model parameters that were altered when fitting the model to the different experimental data sets are shown in Table 1.

#### Extrapolation of the PBPK Model across Alternate Routes of Exposure and Gender of Sprague-Dawley Rats

In the first data set examined, female Sprague-Dawley rats, weighing approximately 190–200 g, were exposed to an iv injection of 5.6 \(\mu\)g TCDD/kg bw (Li et al., 1995). The second data set examined the disposition of TCDD in male Sprague-Dawley rats, weighing approximately 240–290 g, exposed to an iv injection of 9.25 \(\mu\)g TCDD/kg bw (Weber et al., 1993). The concentrations of TCDD in multiple tissues were examined over time in both studies. In addition, both studies reported serum concentrations of TCDD. To compare the model predictions of the blood concentration with the experimental data, the serum concentration was converted to the blood concentration by dividing the serum data by the hematocrit. The data of Li et al. (1995) and Weber et al. (1993) measured TCDD concentrations in both white and brown adipose tissue. These data demonstrate that white adipose tissue accumulates more TCDD than brown does and that the rate of elimination of TCDD is slower in white than brown adipose tissue (Li et al., 1995; Weber et al., 1993). The majority of disposition and pharmacokinetic studies of TCDD and related chemicals have not separated and analyzed the two adipose tissues. Instead, the model is structured such that they are combined into a single adipose-tissue compartment. To compare the model predictions for adipose tissue concentration in Li et al. (1995) and Weber et al. (1993), the experimental data of the white and brown adipose concentrations of TCDD were converted to the total adipose tissue concentration. The conversion was done by...
where $C_{T}$ is the total TCDD concentration of the adipose tissue, while $C_{C}$ and $C_{W}$ are the separate adipose tissue concentrations. The weight ratio of the brown fat to the white fat was assumed to be constant at 0.16 (Roth et al., 1994) and this value was assumed to remain constant with body weight. The hepatic TCDD concentration provided in Weber and coworkers (1993) was also used to examine the dissociation constant of TCDD bound to CYP1A2.

In the previous study (Wang et al., 1997), tissue samples were obtained without exsanguination, while in Li et al. (1995) and Weber et al., (1993) tissue samples were collected after exsanguination. The different preparation of these tissue samples between the different laboratories must be taken into account.

In the data from Wang et al. (1997), the tissue concentration of TCDD includes the concentration in the tissue and in tissue blood. That is,

\[
C_{T} = \frac{C_{W} W_{T}}{W_{T} + W_{TB}}, \quad C_{C} = \frac{C_{W} W_{C}}{W_{W} + W_{TB}}
\]

where $C_{W}$ is the total TCDD concentration in the tissue and tissue blood, $C_{C}$ is the tissue concentration, $C_{TB}$ is the tissue blood concentration, $W_{T}$ is the tissue weight, and $W_{TB}$ is the tissue blood weight. The kidney, liver, and lung are 3 tissues having high-volume fractions of tissue blood (kidney, 16%; liver, 21%; lung, 36%) (ILSI, 1994), and for these tissues the above correction was used to estimate the tissue concentrations in the model. It should be noted that tissue blood concentrations are not equal to the systemic venous/arterial blood concentrations. However, it is accepted that tissue blood concentrations are equivalent to the venous blood concentrations at the site of the vein leaving the tissue prior to mixing with other veins. For all the other tissues examined in this study, the volume fraction of tissue blood is approximately 2–5% of the tissue (ILSI, 1994), and ignoring the sample prepara-

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Parameter Values for PBPK Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sprague-Dawley Rats</td>
<td>(F)</td>
</tr>
<tr>
<td>Initial body weight</td>
<td>250 g</td>
</tr>
<tr>
<td>Equilibrium tissue/blood</td>
<td></td>
</tr>
<tr>
<td>Adipose tissue</td>
<td>100$^a$</td>
</tr>
<tr>
<td>Kidney</td>
<td>6$^a$</td>
</tr>
<tr>
<td>Skin</td>
<td>10$^a$</td>
</tr>
<tr>
<td>Rest of body</td>
<td>1.5$^a$</td>
</tr>
<tr>
<td>Liver</td>
<td>6$^a$</td>
</tr>
<tr>
<td>Lung</td>
<td>6$^a$</td>
</tr>
<tr>
<td>Spleen</td>
<td>5$^a$</td>
</tr>
<tr>
<td>Permeability × area/blood flow rate</td>
<td></td>
</tr>
<tr>
<td>Adipose tissue</td>
<td>0.08$^a$</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.01$^a$</td>
</tr>
<tr>
<td>Skin</td>
<td>0.09$^a$</td>
</tr>
<tr>
<td>Rest of body</td>
<td>0.03$^a$</td>
</tr>
<tr>
<td>Liver</td>
<td>0.35$^a$</td>
</tr>
<tr>
<td>Elimination (h$^{-1}$)</td>
<td></td>
</tr>
<tr>
<td>Urinary</td>
<td>1.0</td>
</tr>
<tr>
<td>Fecal</td>
<td>2.0</td>
</tr>
<tr>
<td>Protein binding (nM)</td>
<td></td>
</tr>
<tr>
<td>TCDD-Ah ($K_{Dah}$)</td>
<td>0.1$^a$</td>
</tr>
<tr>
<td>TCDD-CYP1A2 ($K_{Dah}$)</td>
<td>30$^a$</td>
</tr>
<tr>
<td>Ah receptor concentrations (nM)</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>0.25$^a$</td>
</tr>
<tr>
<td>Skin</td>
<td>0.05$^a$</td>
</tr>
<tr>
<td>Liver</td>
<td>0.35$^a$</td>
</tr>
<tr>
<td>Lung</td>
<td>0.35$^a$</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.1$^a$</td>
</tr>
<tr>
<td>Others</td>
<td></td>
</tr>
<tr>
<td>Basal lever of CYP1A2 (nmol/g liver)</td>
<td>1.6$^a$</td>
</tr>
<tr>
<td>CYP1A2 induction delay (t, h)</td>
<td>0.25$^a$</td>
</tr>
</tbody>
</table>

Note. F, female; M, male.

$^a$Parameter estimation fitted from Wang et al. (1997) model.

$^b$Parameter determined by Kedderis et al., 1991.

$^c$Parameter estimation fitted from Wang et al. (1997) model using data from Li et al. (1995).


$^g$From Poland et al. (1989) for 2,3,7,8-TrCDD.

$^h$Urinary elimination rate constant = 0.0084 × bw$^{0.74}$Vkidney.
tion will not cause a significant deviation in those tissues having such a small fraction of tissue blood volume. Thus, in this model the tissue and tissue blood compartments are separated for kidney, lung, and liver, while in the remaining tissues, tissue blood compartments were not included in the model.

Extrapolation of the PBPK-BBDR Model across Dosage Regimen and Strain of Rat

The PBPK model developed by Wang et al. (1997) was applied to a third data set (Krowke et al., 1989) to predict the tissue concentration of TCDD following an alternate dosage regimen (acute vs. subchronic) in a different strain of rat (Sprague-Dawley vs. Wistar). Krowke and co-workers (1989) exposed male Wistar rats, initially weighing approximately 350 g, to 2 different dosage regimens: (1) an initial sc dose of 25 µg TCDD/kg bw followed by a weekly dose of 5 µg TCDD/kg bw (designated 25-dose in this paper); or (2) an initial subcutaneous dose of 75 µg TCDD/kg bw followed by a weekly dose of 15 µg TCDD/kg (designated 75-dose in the present paper). The concentration of TCDD in multiple tissues was determined over a period of 22 weeks. Initial attempts to fit the model to the 75-dose data set provided unsatisfactory results.

The poor fit of the model to the 75-dose data is most likely due to the physiological and toxicological changes this dosage regimen produced. Animals treated with the 75-dose regimen exhibited a loss of more than 20% of their body weight after 5 weeks and all rats died by 9 weeks. It is likely that the toxicological consequences of this lethal treatment produced large changes in cardiac output, tissue blood flow, percent body fat, percent muscle mass, and numerous other physiological changes. High doses of TCDD also induce hepatic lipid accumulation, decreases in both white and brown adipose tissue triglyceride concentrations, and increases in plasma free fatty acids and triglycerides (Roth et al., 1993). Any or all of these changes could alter the disposition of high doses of TCDD compared to lower and less toxic doses. Because the report by Krowke et al. (1989) does not examine these changes, many of the physiological parameters important for the distribution of TCDD could not be accurately estimated for animals treated with the 75-dose regimen, and subsequently, this data was not further examined. However, at the 25-dose level, animals exhibited little body weight loss and no mortality, and these data were more rigorously examined using the model.

Extrapolation of the PBPK-BBDR Model across Dosage Regimens and from Rats to Mice

Leung et al. (1990b) developed a PBPK model describing the tissue distribution of \(^{125}\text{I}-2\)-iodo-3,7,8-trichlorodibenzo-p-dioxin (ITrCDD) in female C57BL/6J mice (7–10 weeks old, approximately 20 g) exposed to an ip dose of 0.1 nmol ITrCDD/kg. This data set was chosen for PBPK model extrapolation from rats to mice since the tissue concentration of chemical at early time points is known, which is important in determining unique parameters related to mass transfer (Wang et al., 1997). One limitation of this study is that it does not determine CYP1A2 concentrations or induction (Leung et al., 1990b). A dose of 0.1 nmol/kg ITrCDD administered in this study most likely did not result in induction of CYP1A2. While ITrCDD binds to the Ah receptor with equivalent affinity as TCDD (Bradfield et al., 1988; Leung et al., 1990), the equivalent dose of TCDD is only 32 ng/kg, and this dose of TCDD results in minimal induction of CYP1A2 and hepatic sequestration of TCDD (Diliberto et al., 1995). Consistent with this evidence is the finding that there is no indication of hepatic sequestration of ITrCDD in the mice based on the Leung et al. (1990b) data. For these reasons, the mouse model did not include CYP1A2 protein induction.

Prior Estimation of Parameters

Physiological parameters, such as tissue weights and blood flow rates for Sprague-Dawley and Wistar rats and C57BL/6J mice were obtained from the literature (ILSI, 1994). The increases in body weight of female Sprague-Dawley rats over the course of the studies (Li et al., 1995) were estimated from Wang et al., (1997), while the increase in body weight of male Sprague-Dawley rats over the course of the study was obtained from Roth et al. (1994). The growth of the C57BL/6J mice ranges from 19–21 g at 7–11 weeks to 22–24 g at 11–15 weeks (personal communication with Charles River Laboratories, Raleigh, NC). Based on this information, a 20% growth rate for the mice during the period of study (Leung et al., 1990b) was introduced into the model.

Urinary clearance is not a critical parameter for rats, since the cumulative elimination via this route is much smaller than that through fecal elimination (Diliberto et al., 1993, 1996; Gasiewicz et al., 1983). The expression, \(0.0064 \times bw^{0.89} \text{ml/min}\), was used to calculate the urinary elimination constant for both Sprague-Dawley and Wistar male rats as determined by Roth et al. (1994). The urinary clearance from a 250 g female Sprague-Dawley rat was fitted from the data of Wang et al. (1997) and allometric scaling was used to estimate the urinary elimination rate constant for the Li et al. (1995) data (see Table 1). In contrast to rats, urinary elimination for mice can account for 10 to 20% of total dose, and therefore, the elimination constants of biliary and urinary excretion for mice were estimated based on experimental data (Birnbaum, 1986). It should be noted that the model does not contain a sub-model describing the elimination of the metabolites of TCDD in either urine or feces for either mice or rats.

Other parameters, such as the membrane permeability, partition coefficients, binding affinity to the AhR, induction of CYP1A2, and the apparent dissociation constant of TCDD for CYP1A2, were fit to the data (Wang et al., 1997). The iv injection was simulated with a function described below (Bischoff, 1967):

\[
g(t) = 30A(\lambda t(1 - \lambda t))^{(\lambda t - 1)},
\]

where \(g(t)\) describes the bolus infusion of the chemical, \(U(\lambda t - 1)\) is a step function, and \(\lambda\) represents the reciprocal pulse width of \(g(t)\). At \(t = 1\) the pulse is cut off. The faster the input of the dose, the larger \(\lambda\) becomes. This function was chosen based on the success of previous models of iv injections that incorporated this function (Bischoff, 1967).

The ip and sc injections were simulated using the same \(g(t)\) function. Instead of directly entering the venous blood compartment of the injection, the input dose was assumed to enter some depot before entering the blood circulation,

\[
r = \text{dose } g(t)
\]

\[
\frac{dm_1}{dt} = r - k_{a1} m_2
\]

where \(r\) is the rate of input to the depot, \(m_1\) is the amount of TCDD at the depot, and \(k_{a1}\) is the rate of TCDD entering the blood circulation.

The induction of CYP1A2 and other proteins by TCDD is not an instantaneous event. Experimental data indicate that there is a lag period following exposure to TCDD before measurable increases of CYP1A2 and other proteins are detected (Santostefano et al., 1998). These time lags range from 3–6 h, depending on the study. This time delay is due to a number of biological processes, such as the time it takes to bind and activate the Ah receptor, for the activated receptor to bind to DNA and increase production of mRNA, and the time it takes to synthesize protein from mRNA. In addition, the delay may be related to the ability to detect small changes in protein concentrations. The delay in CYP1A2 induction was simulated using a series reaction,

\[
\tau \frac{dr_{cyo1a2}}{dt} = r_{CYPA1A2(j-1)} - r_{CYPA1A2}, \quad j = 1, 2, 3
\]

where \(\tau\) is the delay time, and \(j\) is the number of compartments or reactions required to simulate the delay in CYP1A2. In the original model, the param-
eters $\tau$ and $j$ were fitted to data from experiments using an oral route of exposure (Santostefano et al., 1998) and were estimated by the model at 0.25 and 3 h, respectively. In the present study, the data from Weber et al. (1993) were used to fit these delay parameters and the resulting parameters fits were used for the other data sets examined.

Model simulations were conducted using ACSL Tox (Pharsight Co., 1998, Mountain View, CA).

RESULTS

Model Prediction of TCDD Distribution in Male and Female Sprague-Dawley Rats

Figure 1 presents the model predictions and the experimental data for the tissue distribution of TCDD in female Sprague-Dawley rats following an iv injection of 5.6 $\mu$g TCDD/kg (Li et al., 1995). One-day postexposure, TCDD concentrations were highest in the liver, followed by adipose tissue, with the lowest concentrations found in blood. The PBPK model developed by Wang and coworkers (1997) accurately predicts the time course of TCDD tissue concentration in the liver, adipose tissue, skin, blood, muscle (rest of the body), lungs and kidneys from the data of Li et al. (1995) (Fig. 1).

The spleen concentration of TCDD predicted by the model (data not shown) is approximately 3- to 4-fold lower than the experimental data reported by Li et al. (1995). Studies from other laboratories indicate that concentration of TCDD in the spleen is similar to TCDD concentrations in the thymus (Diliberto et al., 1995; Wang et al., 1997). However, in the study by Li et al. (1995), the spleen concentration of TCDD is approximately 3- to 5-fold greater than that found in the thymus at all time points. The difference between the spleen concentration data from Li et al. (1995) and other data sets remains uncertain.

The model fitted parameter values are presented in Table 1. A few of the parameter values were altered when fitting the data of Li et al. (1995), compared to values fitted using the data of Wang et al. (1977). Small changes (less than 40%) in the parameter estimates of the model for the equilibrium kidney/blood, skin permeability $\times$ area/blood rate, urinary and fecal elimination rates, and CYP1A2 $K_{DA2}$ were required to fit the model to the Li et al. data (1995). Larger alterations in the rest of the body permeability $\times$ area/blood and in the time delay for CYP1A2 induction were required to fit the model to the Li et al. (1995) data. Using the same parameters used to fit the Wang et al. (1997) data, with the exception of the time delay parameters, changed the predictions by approximately 10% or less compared to the parameters fitted to the Li et al. (1995) data. The time delay used to fit the Li et al. (1995) data was based on time-delay parameters for the Weber et al. (1993) data and was 6 times greater than the delays used to fit the Wang et al. (1997) data.

Figure 2 presents the experimental data and the PBPK model predictions for the tissue distribution of TCDD in the liver, adipose tissue, skin, and blood of male Sprague-Dawley rats exposed to an iv injection of 9.25 $\mu$g TCDD/kg bw (Weber et al., 1993). The highest concentrations of TCDD were found in liver, followed by the adipose tissue. Lowest concentrations were found in the blood. The PBPK model accurately predicts the TCDD concentration in the liver, adipose tissue, skin, kidneys and spleen. Estimates of the fitted parameters were different from the original model fits to the Wang et al. (1997) data for adipose tissue and kidney equilibrium tissue/blood, adipose tissue, skin and rest of the body permeability $\times$ area/blood rate, $K_{DA2}$, and urinary and fecal elimination rates. Most differences in parameter estimates between models using the
Weber et al. (1993) data set and the Wang et al. (1997) data set were within 640% of the original model fits (Wang et al., 1997). Noted exceptions were the time delay and the permeability \( \times \) area/blood rate for the rest of the body. The time delay for enzyme induction was 6 times greater than that estimated in the original model. The permeability \( \times \) area/blood rate for the rest of the body compartment was 2.2 times those fitted to the Wang et al. (1997) data.

Pulmonary concentration of TCDD observed in Weber et al. (1993) at the very early time points is extremely high, reaching 0.7 nmol/g (Fig. 2), and decreasing to 0.003 at 200 h. In contrast, the PBPK model predicts peak concentrations at least one order of magnitude lower and a more rapid elimination rate in the lung (Fig. 2). It should be noted that the experimental data of Weber is not consistent with experimental data from Wang et al. (1995) and Li et al. (1995), in that these later data sets have lower peak lung concentrations with more rapid elimination from the lung, consistent with the model predictions. One plausible explanation of these differences is that, in the Weber et al. (1993) study, the TCDD in the dosing solution was in an emulsion and may have come out of solution. If this were the case, then TCDD particles would have been trapped in the lungs resulting in higher than expected concentrations and slower than expected elimination.

As mentioned by the authors (Weber et al., 1993), the basal CYP1A2 concentration in the liver is 1.78 nmol CYP1A2/g liver, which is consistent with that reported from our laboratory (Kedderis et al., 1991) for male F344 rats (1.6 nmol CYP1A2/g liver). Therefore, in the present study, the basal CYP1A2 concentration was set at 1.6 nmol CYP1A2/g liver (Table 1). Information on the basal CYP1A2 concentration allows a unique fitting of the binding affinity of TCDD to CYP1A2, based upon the TCDD concentration in the liver prior to CYP1A2 induction. Using the hepatic tissue concentrations from Weber et al. (1993), the dissociation constant of TCDD binding to CYP1A2 is estimated at 35 nM. This result is also consistent with our previous study (Wang et al., 1997). In addition, using the same information, the delay in CYP1A2 induction was estimated, where \( \tau \) in Equation 3 was determined to be equal to 1.5 h and \( j \) equals 3. The value for \( \tau \) was greater when fit to the data from Weber et al. (1993) when compared to the value estimated when fit to the data of Wang et al. (1997) (Table 1). Figure 2 also presents the PBPK model prediction of the liver concentrations compared to the experimental data at early time points after dosing and prior to full induction of CYP1A2. The agreement between the model predictions and the experimental data supported the parameter values related to the TCDD:AhR interaction and protein induction previously reported in other studies (Kedderis et al., 1991; Wang et al., 1997).

**Model Prediction of TCDD Tissue Distribution in Male Wistar Rats**

Figure 3 presents the experimental data and the model predictions for the distribution of TCDD in liver, adipose tissue, and kidneys of male Wistar rats treated with a single subcutaneous dose of 25 \( \mu \)g TCDD/kg bw followed by a weekly maintenance dose of 5 \( \mu \)g TCDD/kg bw (Krowke et al., 1989). In the study by Krowke et al. (1989), 2 animals/time points were examined for tissue concentrations of TCDD. The tissue concentrations from the 2 male Wistar rats per time point are shown in Figure 3, as reported in Krowke et al. (1989). The concentrations of TCDD in the adipose tissue, liver, and kid-
neys were rather constant over the course of the study. The prediction obtained from the model (Wang et al., 1997) reasonably agrees with the experimental data (Fig. 3). Minimal changes in parameter estimates were required to fit the model to the Krowke et al. (1989) data compared to model fits using the Wang et al. (1997) data. The parameter values that differed from those fitted to the Wang data were the permeability area/blood rate for adipose tissue, skin, and the rest of body compartment, $K_{DA2}$, urinary elimination rate, and the CYP1A2 induction delay, which was based on Weber et al. (1993) data. Most changes were within 40% of the parameter estimate fitted to the Wang et al. (1997) data. Similar to the Li et al. (1995) and Weber et al. (1993) data sets, the rest of the body compartment permeability × area/blood rate was 2.2 times that fitted to the Wang et al. (1997) data. The rest of the body compartment predominately consists of muscle and bone. Because these tissues have low concentrations, it is difficult to accurately fit permeability × area/blood rate. In addition, this parameter has little effect on the overall model and altering the parameter from 0.03 to 0.08 only changes estimates of muscle concentration from 6 to 10%.

Model Prediction of ITrCDD Tissue Distribution in Female C57BL/6J Mice

Figure 4 shows the experimental data and PBPK model predictions for the tissue distribution of ITrCDD in the liver, adipose tissue, kidneys, muscle and blood in female C57BL/6J mice exposed to an sc dose of 0.1 nmol ITrCDD/kg (Leung et al., 1990b). In the experimental data, the concentration of ITrCDD in the adipose tissue is higher than in the liver, followed by kidney, muscle, and blood. The liver/adipose tissue concentrations in the mice were ≤0.4 at all time points examined, indicating that hepatic sequestration was not occurring and that induction of CYP1A2 was unlikely in these animals (DiLiberto et al., 1997). The model predictions were consistent with these experimental results (Fig. 4). Mouse specific organ and body weights and physiological parameters such as blood flow rates and cardiac output were used to fit the ITrCDD data. Several of the fitted parameters were altered compared to the model using the Wang et al. (1997) data. Equilibrium tissue/blood values increased by 50–100% for adipose tissue, kidney, skin and rest of the body when using the mouse data for ITrCDD (Table 1). The permeability × area/blood rate increased by 50% for adipose tissue and decreased by 23% for skin. Urinary and fecal elimination rate constants were also slightly altered (Table 1). AhR binding affinity was set equivalent to that with TCDD in the rat. The basal concentrations of CYP1A2 and $K_{DA2}$ in the ITrCDD model were altered by less than 15%. Considering that the experimental data is for another chemical and in a different species, the model predicts the data well with these limited changes in parameter estimates.

DISCUSSION

The PBPK model developed, based upon dose-response and time course data following a single oral dose of TCDD to female Sprague-Dawley rats (Wang et al., 1997), accurately predicts the concentration of TCDD in multiple tissues (Figs. 1–4) obtained from different laboratories under varying conditions (Krowke et al., 1989; Leung et al., 1990b; Li et al., 1995; Weber et al., 1993). With few exceptions, the fitted parameter estimates were similar between the studies and the slight differences are within measurement error or within in-
individual variability. One exception is the time delay constant used in the induction of CYP1A2. This difference can be attributed to a combination of different routes of exposure used between studies, a limited understanding of the biological mechanism of the delay, and a lack of data examining these issues. The time-delay constant represents an attempt to mathematically describe this biological phenomena given the limited data available, and as such, is an empirical fit to the pharmacokinetic data. It should be noted that without the time delays, the model does not adequately predict the time course for induction or tissue concentrations at the early time points. Other models also include a time delay for protein induction and these values are similar to those used in the present exercise.

In our previous study (Wang et al., 1997), membrane permeability was determined based on the early time points of tissue distribution following an oral dose. In the study by Weber et al. (1993), the time course for tissue distribution of TCDD was obtained following an iv injection. When the model was fit to the Weber et al. (1993) data, some of the membrane permeability parameters were altered compared to those used to fit the Wang et al. (1997) data. The differences in the estimates of these parameters may be due to the different route of exposure and the dosing vehicle used. Following an oral dose of a chemical in a corn oil vehicle, the rate of tissue uptake is controlled by the mass transfer from the GI tract to the lymph phase, diffusion from the lymph to the blood, and diffusion across the tissue membrane. Following an iv dose, the rate of tissue uptake is determined by the rate of membrane transfer from the emulsion to the blood phase and convection across the membrane. For sc and ip routes of exposure, the input dose was assumed to enter some depot before entering the blood. The estimated values for the membrane permeability in these studies are empirical estimations affected by the differences in dosing solutions and routes of administration. Therefore, when fitting the model to the different data sets (Table 1), the small variability observed for estimates of the permeability-area product was expected. In addition, attempts to measure TCDD tissue:blood partition coefficients from in vitro experimental systems vary by greater than 50% in fat, liver, kidney, and muscle (Murphy et al., 1995). These experimental values vary as much as the fitted values derived from different data sets. These findings indicate that small changes in parameters relating to permeability may still provide reasonable estimates of the true values.

The difficulties in determining the unique parameter values related to receptor-ligand binding and protein induction were addressed in our previous study (Wang et al., 1997). Using sensitivity analysis, Evans and Andersen (2000) demonstrate that CYP1A2 concentrations and the TCDD dissociation constant control liver concentrations of TCDD, in part, for CYP1A2. If the basal level of hepatic CYP1A2 is known, the apparent dissociation constant of TCDD to CYP1A2 can be uniquely determined using the experimental data of TCDD concentration in the liver prior to CYP1A2 induction. In the present exercise, only one data set determined basal CYP1A2 concentrations. In the Weber et al. (1993) study, basal CYP1A2 was 1.78 nmol/g liver, which was similar to earlier determinations of basal CYP1A2 of 1.6 nmol/g liver from our laboratory (Kedderis et al., 1991). Based on the similarity of the basal CYP1A2 concentrations between these two studies, basal concentrations of CYP1A2 for rats were set using the Kedderis et al. (1991) data, and the apparent dissociation constant of CYP1A2 was then fit to the separate kinetic data.
sets. The fits of the apparent dissociation constants varied slightly between the data sets. These differences in the estimates of the apparent dissociation constant may be due to differences in the basal and inducible CYP1A2 concentrations between the studies. In addition, CYP1A2 metabolizes a number of endogenous and exogenous compounds such as estradiol and porphyrins (Lambrecht et al., 1992). The presence of these chemicals may alter the estimation of the apparent dissociation constant of TCDD for CYP1A2. The concentrations of these chemicals are expected to vary between studies due to differences in gender, diet, and perhaps, other environmental factors. The potential differences in CYP1A2 and the presence of other ligands for this protein may also influence the estimation of the apparent dissociation constant and result in different estimates, based on the experimental data or the species examined.

The present study also shows that the model for rats describes the tissue concentration in mice with suitable scaling of model parameters (Fig. 4, Table 1). Previous experimental data from female Sprague-Dawley rats (Wang et al., 1997) showed that the liver/adipose tissue concentration ratio of TCDD is about 0.9 on day 3 following a single oral dose of 0.01 μg TCDD/kg. The results obtained from mice show that the liver/adipose tissue concentration ratio of TCDD is ≤0.4 following a single oral dose of 0.1 nmol ITrCDD/kg (Leung et al., 1990b). In CYP1A2 knockout mice, similar liver/adipose concentrations were observed for TCDD (Diliberto et al., 1995), suggesting that there is no induction of CYP1A2 in the mice treated with low doses of ITrCDD (Leung et al., 1990b). Although these chemicals are structurally similar, pharmacokinetic and pharmacodynamic differences have been observed (Poland et al., 1989a,b).

Our previous study (Wang et al., 1997) clarified that the partition coefficients obtained from studies by Leung and coworkers (1989, 1990a,b) are defined based on the free blood concentration, due to an inappropriate handling of the mass balance equation. Converting the partition coefficient for the adipose tissue and the liver reported by Leung et al. (1990b) defined on the total blood concentration, the equilibrium distribution ratio of the adipose tissue to blood and liver to blood is 150 and 5 respectively (Wang et al., 1997). Using these values in the current study, the model accurately predicts across species and ligand (Table 1, Fig. 4).

PBPK models are tools that can be used for data analysis, study design, and species extrapolation. For risk assessment purposes, these models can be used for extrapolation of animal data to humans. Prior to using this model for animal to human extrapolation, a number of uncertainties must be more rigorously examined. The distribution of TCDD is controlled in part by the its binding to the Ah receptor and induction of CYP1A2, the hepatic binding species. There are polymorphisms in the human Ah receptor, which result in expression of AhRs with binding affinities ranging over a factor of 20 (Micka et al., 1997). There is limited data suggesting that TCDD and related chemicals are sequestered in hepatic tissue (Carrier et al., 1995; Thoma et al., 1990). However, the dose-response relationship for hepatic sequestration in humans remains uncertain. Future efforts should be made to collect data on AhR polymorphisms and CYP1A2 interactions with TCDD, for use in extrapolation of these models to humans.

The present study demonstrates that the PBPK model for the tissue distribution of TCDD in female Sprague-Dawley rats developed by Wang and coworkers (1997) predicts the experimental data of TCDD distribution in multiple tissues of rats obtained from different laboratories under different conditions, with minimal changes in fitted parameters. In addition, this model was able to predict the tissue distribution of a chemical structurally related to TCDD in mice. This study provides further confirmation of the potential use of physiological modeling in understanding pharmacokinetics and pharmacodynamics in different species.

APPENDIX

Mathematical Derivation

The derivation of the mathematical expressions were shown in a previous publication (Wang et al., 1997).

Total body weight change. For female SD rats, \( W_t = W_{t_0} (1+0.41\text{ time/1402.5 } + \text{ time}) \), where \( W_{t_0} \) is the initial body weight (g), and the unit of time is h.

Expressions for total tissue concentration in tissue subcompartment. For extrahepatic tissues, the total tissue concentration can be expressed by Equations A1, A2, or A3:

\[
C_T = \frac{N_T C_{Tr} + \frac{Ah_T C_{Tr}}{K_{Da} + C_{Tr}}}{(A1)}
\]

\[
C_T = P_T C_{TBf} + \frac{Ah_T C_{Tr}}{K_{Da} + C_{Tr}} (A2)
\]

\[
C_T = P_C C_{TBf} + \frac{Ah_T C_{Tr}}{K_{Da} + C_{Tr}} (A3)
\]

In the liver, the term of \( \frac{C_{AB} C_{Llf}}{K_{Da2} + C_{Llf}} \) needs to be added to Equations A1, A2, or A3.

Mass balance equation if there is linear binding of tcdd to plasma proteins. The general mass balance equation should be expressed as Equations A4 and A5:

\[
\frac{dW_{TB} C_{TB}}{dt} = Q_T (C_B - C_{TB}) - PA (C_{TBf} - C_{Tr}) (A4)
\]

\[
\frac{dW_{L} C_{L}}{dt} = PA (C_{TBf} - C_{Tr}) (A5)
\]

For those tissues without strong specific binding, such as adipose tissue, Equations A4 and A5 become...
Then, for a flow-limited case,
\[
\frac{dW_T}{dt} = Q\left(C_B - C_T\right) - PA\left(C_{TB} - C_T/P\right)
\]  
(A6)

and for a membrane-limited case,
\[
\frac{dW_T}{dt} = PA\left(C_{TB} - C_T/P\right)
\]  
(A7)

and

\[
\frac{dW_T}{dt} = Q\left(C_B - C_T\right)
\]  
(A8)

where,
\[
C_{TB} = \frac{C_B + C_PA/Q}{1 + PA/Q}
\]

or

\[
C_{TB} = \frac{C_B + C_PA/Q}{1 + PA/Q}
\]

(A9)

and

\[
\frac{dW_T}{dt} = PA\left(C_{TB} - C_T/P\right)
\]  
(A10)

\[
C_{TB} = \frac{C_B + C_PA/Q}{1 + PA/Q}
\]

\[
C_{TB} = \frac{C_B + C_PA/Q}{1 + PA/Q}
\]

\[
C_{TB} = \frac{C_B + C_PA/Q}{1 + PA/Q}
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\[
C_{TB} = \frac{C_B + C_PA/Q}{1 + PA/Q}
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C_{TB} = \frac{C_B + C_PA/Q}{1 + PA/Q}
\]

\[
C_{TB} = \frac{C_B + C_PA/Q}{1 + PA/Q}
\]

CYP1A2 induction
\[
\frac{dW_{Li}}{dt} = \left(S(t)K_0 - K_2C_{A2}\right)W_{Li}
\]  
(A11)

at
\[
t = 0, C_{A2} = C_{A2BS}
\]

also,
\[
K_0 = K_2C_{A2BS}
\]

where
\[
S(t) = 1 + \ln C_{A2}\left(\frac{C_{A2}}{IC_{A2}}\right)^{h}\]

is the stimulation function.

Oral or ip absorption rate. The oral or ip absorption rate was simplified as \(-kst \times a\), where \(a\) is the amount in the depot or GI tract.

Nomenclature

A, fraction delivered by lymph
Ah, Ah receptor concentration (nM)
Bio, bioavailability
C, concentration (nmol/g)
Dor, oral dose
F, volume fraction

H, Hill coefficient
IC_{A2}, Michaelis-Menten constant of CYP1A2 induction (nM)
In_{A2}, maximum induction fold over basal synthesis rate of CYP1A2
K_{A0}, CYP1A2 basal synthesis rate (nmol/g/h)
K_{A2}, CYP1A2 degradation rate (nmol/g/h)
K_{AB}, linear binding constant of TCDD to plasma proteins
K_{Abs}, absorption rate from lumen (1/h)
K_{DAB}, dissociation binding constant of TCDD – Ah (nM)
K_{DAB2}, dissociation binding constant of TCDD – CYP1A2 (nM)
C_{A2-TCDD}, amount of Ah receptor occupied by TCDD, AhLi
CLifKDAh + CLif
K_{a}, linear binding of TCDD to any constituent in the intracellular domain
K_{S}, distribution ratio of TCDD in lipid/water in the intracellular domain
K_{S}, stomach emptying rate (1/h), or ip absorption rate constant
K_{T}, elimination rate of TCDD from the tissue (1/h)
P, equilibrium distribution ratio
PA, permeability \times area (ml/h)
Q, blood flow rate (ml/h)
W, weight (g)

Subscripts

A2, CYP1A2
A2BS, CYP1A2 basal concentration
Ah, Ah receptor
B, blood
F, free concentration
 Fat
K, kidney
Lip, lipid content
Li, liver
Lu, lung
Lum, lumen
Nb, linear binding constituent
S, skin
Sp, spleen
St, stomach
Re, the rest of the body
T, tissue
T, total
W, water

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