



A PHARMACOKINETIC ANALYSIS OF INTERSPECIES EXTRAPOLATION IN DIOXIN RISK ASSESSMENT

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ABSTRACT

This study entails a pharmacokinetic analysis of the relationship between the external dose of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (dioxin, TCDD) and resulting concentrations of TCDD in internal tissues and organs of humans and rodent species. The methodology is based on the development and testing of physiologically based pharmacokinetic models for several rodent species and humans. The results indicate that the relationship between the external dose of TCDD and resulting TCDD concentrations in liver and adipose tissue of humans and various species of rats and mice can vary by as much as 725 fold, illustrating that humans and experimental animals differ considerably in their ability to convert external dosages of dioxin to tissue concentrations. Interspecies scaling factors are reported to express the differences in tissue concentrations of dioxin between mice, rats and humans in response to an equivalent external dose. The significance of these findings for conducting human cancer and ecological risk assessments is discussed. It is recommended that pharmacokinetic differences be considered explicitly in risk estimation, while separately recognizing interspecies differences in pharmacodynamics (sensitivity).
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INTRODUCTION

In human health and ecological risk assessments of environmental contaminants it is common practice to extrapolate toxicological responses observed in animal bioassays to those in humans (Albert, 1989; Fishbein, 1986; Gaylor et al., 1993). In most cases concerned with contaminants at environmental concentrations, this extrapolation involves (i) an interspecies extrapolation, where the effects observed in test organisms are extrapolated to humans or other organisms, and (ii) a high-to-low-dose extrapolation, where the effects observed at high "experimental" doses are extrapolated to the low "environmental" doses to which humans and organisms are typically exposed. The interspecies extrapolation is often performed by scaling the dose administered to the test organism to body weight, resulting in what we will refer to as an "external dose", typically expressed in units of milligrams of chemical per kilogram of organism body weight per day. If the chemical is recognized as a

carcinogen, human cancer risk assessment often assumes that the tumor incidence observed in the test organism is similar to that in humans when the dose is scaled to body weight, i.e. expressed as the same "external dose", causing all uncertainty in the risk assessment to be accounted for in the high-to-low-dose extrapolation. If the chemical is not a carcinogen, the risk assessment, which is then referred to as a hazard assessment, involves the comparison of the external dose (to which humans are exposed) to the no-observable-effects dose in the test organism, assuming that the response observed in the test organism is similar to that in humans if the dose is scaled to body weight, i.e. expressed as the same "external dose". Safety or uncertainty factors are introduced to safeguard against potential errors and uncertainty in this and other assumptions. For example, the EPA recommends that an uncertainty factor between 1 and 10 is used to account for potential errors and uncertainties in interspecies extrapolation, while other uncertainty factors have been recommended to account for other uncertainties (U.S. EPA, 1989). However, there is little knowledge of what the magnitude of these errors in assumption might be. As a result, the safety factors are likely to be chosen arbitrarily, affecting the credibility of the assessment.

The relationship between external dose and cancer risk involves two components, i.e. "dosimetry", which determines the concentrations in the internal tissues that are reached given a certain external dose, and "sensitivity", controlling the extent of effect (e.g. tumor formation for TCDD) at the target tissue concentration. In this paper we will investigate the differences in relationships between the "external dose" and resulting tissue concentrations ("effective dose") among various rodent species and humans. Differences in the sensitivity of target organs and tissues to the chemical (here defined as the level of effect observed at a particular concentration in the target organ or tissue) are also important factors affecting the risk assessment and should be investigated separately. Recently, the relative sensitivity of rat and human tissues to TCDD was investigated by Aylward et al. (1996). In this study we will focus on pharmacokinetic factors affecting the TCDD risk assessment, but we will briefly discuss the importance of combining sensitivity and pharmacokinetic considerations. Pharmacokinetically controlled differences in the response of various organisms to a certain dose (in mg/day) of a chemical substance is often accounted by expressing the dose as an external dose in units of mg/kg/day. The U.S. EPA and FDA have proposed a cross-species scaling factor equivalent to $\text{mg/kg}^{0.75}/\text{day}$ to account for allometric differences in the pharmacokinetics and sensitivity among species in absence of adequate information on pharmacokinetic and sensitivity differences between species (U.S. EPA, 1992). Body surface area scaling has also been suggested as a method for extrapolating differences in pharmacokinetics between species (Voisin et al., 1990). The use of physiologically based pharmacokinetic (PB-PK) models has frequently been cited as a preferred method for characterizing the ability of different organisms to convert external dosages of chemical substances to tissue concentrations. Traditionally, PB-PK models have not been used in a regulatory framework for human risk assessments of dioxin. In this study, we present PB-PK models for TCDD in humans, rats and mice. The models that we present are modified or adapted from PB-PK models reported in other studies. The models are tested against observed data and used to determine the relationship between external dose and resulting tissue concentrations in various organisms. The main goal of this analysis is to determine the potential errors that can be

made in a risk assessment when equivalents of the “external dose” are chosen as the basis for extrapolating a toxicological response from a test organism to humans or other species. Although the study is limited to one chemical, i.e. 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), the results are believed to be applicable to the methodology of risk assessment of many environmental contaminants.

METHODOLOGY

The methodology used to investigate the relationship between the “external dose” and internal concentrations of TCDD in various tissues and organs consists of three parts. First, we formulate the relationship between external dose and tissue concentration in humans, rats, and mice in terms of physiologically based pharmacokinetic (PB-PK) models. Then, we verify the models against observed data to evaluate the models’ ability to represent the internal pharmacokinetics of TCDD in humans and rodent species. Finally we conduct model simulations of the relationship between the external dose and concentrations of TCDD in the liver and adipose tissue of humans and rodents at dosage levels relevant to TCDD risk assessment. The essence of the third part of the study is to reconstruct the pharmacokinetics of TCDD in the bioassays that have been used as the basis for TCDD risk assessment. To describe the bioaccumulation and internal distribution of dioxin in humans and several rodent species, we have modified existing physiologically-based pharmacokinetic (PB-PK) models. These models depict metabolic and physiological processes in various compartments of the body, and are used to mathematically describe the biological fate of chemicals from the point of exposure to the target organs and tissues. In the parameterization of these models, we have relied on the physiological and metabolic input data that have been shown to be successful in the work of previous authors. Although some of the input parameters may have been derived through model calibration, we have not further calibrated these models in this study. It should be noted that there have been some significant advances in pharmacokinetic modelling, especially in rodents (Andersen et al. 1993, Kohn et al. 1995). However, for the purpose of this analysis, the somewhat simpler models used in this study are adequate and produce results that are in agreement with the more complex models. We coded separate programs for the human and rodent models to reflect differences in the mass balance equations, although the models are structurally quite similar.

Model Construction:

The *human PB-PK model* used in this study represents a modification of the equations presented in Kissel and Robarge (1988), which are based on Patterson and Mackay (1987). The most important modification involves the expressions for the gastro-intestinal uptake of TCDD to reflect recent findings regarding the mechanism of gastro-intestinal uptake of very hydrophobic substances (Gobas et al., 1993). The modelling framework consists of several physiological compartments representing body tissues or organs (Figure 1). Each compartment is internally homogenous with respect to the concentration of TCDD, and tissue concentrations are in equilibrium

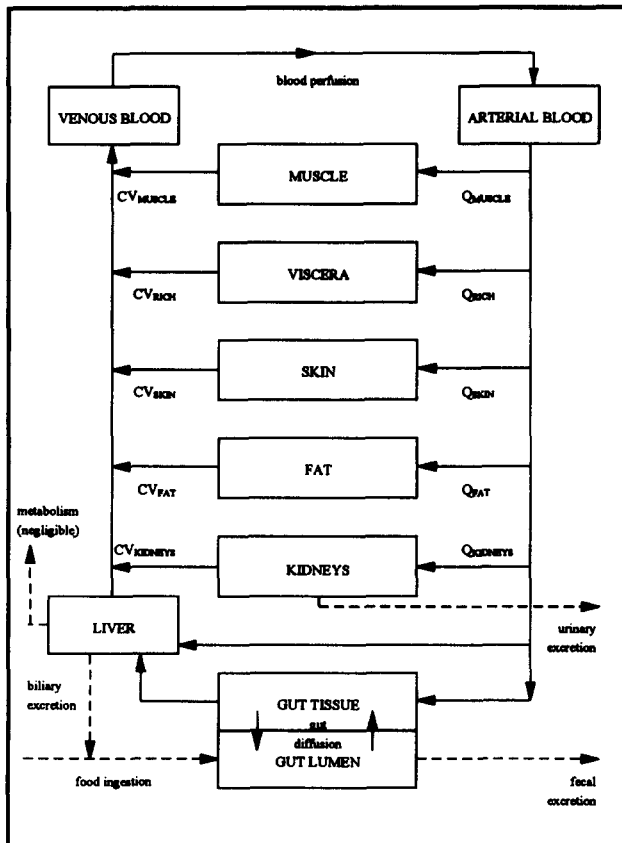


Figure 1. Diagram of the Human Physiologically-Based Pharmacokinetic Model

with the blood (Kissel and Robarge, 1988). Blood flows and tissue: blood partition coefficients predominantly determine the rate at which TCDD is transported from one compartment to another. Intake of dioxin is exclusively through ingestion of food items. This is an appropriate assumption since more than 98% of TCDD exposure to humans occurs through food consumption (Travis and Hattemer-Frey, 1991). Dermal absorption and air inhalation were ignored as significant routes of dioxin exposure. For each compartment, a set of nonsteady-state equations describes the distribution of the chemical (Appendix A). These mass balance equations were solved numerically using an Euler type numerical integration. Physiological parameters for the model (i.e. compartment volumes, densities, blood perfusion rates, and tissue: blood partition coefficients) were obtained from Kissel and Robarge (1988) (Table 1). We assumed metabolic transformation of TCDD to be negligible based on studies by Ryan (1986). As the human model is used to make assessments for conditions associated with background exposures, binding to specific hepatic proteins, as is observed in rats, was not considered. The model also did not consider diffusion limited tissue absorption, as is done by Andersen et al. (1993) for rodents, because (i) there are difficulties with the parameterization of such a model (for all species), (ii) parameterization of the PBPK model, whether it is a

flow or a diffusion limited model, ultimately relies on calibration with an empirical data set and (iii) both flow and diffusion limited models result in similar steady-state concentration values, which are the focus in lifetime excess risk assessments. Finally, the effect of diffusion limitation on the time dependence of dioxin tissue concentrations in humans is considerably smaller than that in the smaller rodents due to the smaller rate of dioxin elimination per unit of body weight. The model further ignores changes in body weight and body composition throughout a human's

Table 1. Model Parameters for the Human PB-PK Model

Source: Kissel and Robarge (1988)

Parameter	Abbreviation	Units	Value
Body Weight	BW	(kg)	70
<u>Compartment Volumes</u>			
Gut Tissue	$V_{OUTTISSUE}$	(m^3)	0.0012
Gut Lumen	$V_{OUTLUMEN}$	(m^3)	0.0010
Liver	V_{LIVER}	(m^3)	0.0015
Blood	V_{BLOOD}	(m^3)	0.0026
Adipose Tissue	V_{FAT}	(m^3)	0.0134
Skin	V_{SKIN}	(m^3)	0.0023
Muscle Tissue	V_{MUSCLE}	(m^3)	0.0262
Richly Perfused Tissue	V_{RICH}	(m^3)	0.0026
Kidneys	$V_{KIDNEYS}$	(m^3)	0.0002
<u>Blood Flow</u>			
Gut Tissue	Q_{GUT}	(m^3/h)	0.0603
Liver	Q_{LIVER}	(m^3/h)	0.0837
Adipose Tissue	Q_{FAT}	(m^3/h)	0.0178
Skin	Q_{SKIN}	(m^3/h)	0.0120
Muscle Tissue	Q_{MUSCLE}	(m^3/h)	0.0540
Richly Perfused Tissue	Q_{RICH}	(m^3/h)	0.0909
Kidneys	$Q_{KIDNEYS}$	(m^3/h)	0.0568
Cardiac Output	Q_{BLOOD}	(m^3/h)	0.3151
<u>Partition Coefficients (Ratio of Fugacity Capacity in Tissue to that in Blood)</u>			
Gut Tissue	$R_{GUT} = Z_{GUT} / Z_{BLOOD}$	(unitless)	10
Liver	$R_{GUT} = Z_{LIVER} / Z_{BLOOD}$	(unitless)	25
Adipose Tissue	$R_{GUT} = Z_{FAT} / Z_{BLOOD}$	(unitless)	300
Skin	$R_{GUT} = Z_{SKIN} / Z_{BLOOD}$	(unitless)	30
Muscle Tissue	$R_{GUT} = Z_{MUSCLE} / Z_{BLOOD}$	(unitless)	4
Richly Perfused Tissue	$R_{GUT} = Z_{RICH} / Z_{BLOOD}$	(unitless)	10
Kidneys	$R_{GUT} = Z_{KIDNEYS} / Z_{BLOOD}$	(unitless)	7
Bile	$R_{GUT} = Z_{BILE} / Z_{BLOOD}$	(unitless)	0.5
<u>Other Constants</u>			
Urinary rate	G_{URINE}	(m^3/h)	4.17E-05
Biliary excretion	G_{BILE}	(m^3/h)	2.08E-05
Metabolic transformation constant	k_M	(1/h)	0
Henry's Law Constant	HLC	($Pa \cdot m^3/mol$)	3.3
TCDD molecular weight	MW	(g/mol)	322
Dietary efficiency	ϵ	(unitless)	0.9
Fugacity capacity of blood	Z_{BLOOD}	($mol/Pa \cdot m^3$)	6061

life-time because of a lack of relevant physiological input parameters, lack of data regarding life-time TCDD dosages and because human cancer risks are most relevant when considered over a lifetime, much of which is spent in adulthood.

The *rodent PB-PK model* used to describe the bioaccumulation and internal distribution of TCDD in rodents is similar to the human model (Figure 2). The mass balance equations and model parameters in the model were obtained from Leung et al. (1988, 1990b), and are presented in Appendix B and Table 2. The most important departure from the human model is the inclusion of the specific binding of TCDD to TCDD-binding proteins in the rodent liver and in blood, which is observed at the high dosage levels used in rodent bioassays (Mills et al., 1992). At high doses of TCDD, certain proteins are induced in rats, mice, and other rodents which have the effect of sequestering more TCDD in the liver than would result from simple physico-chemical partitioning. Specifically, the model considers two liver proteins, one a high affinity, low capacity cytosolic protein, and the other a low affinity, high capacity microsomal protein (Leung et al., 1988). The induction of these proteins was not considered

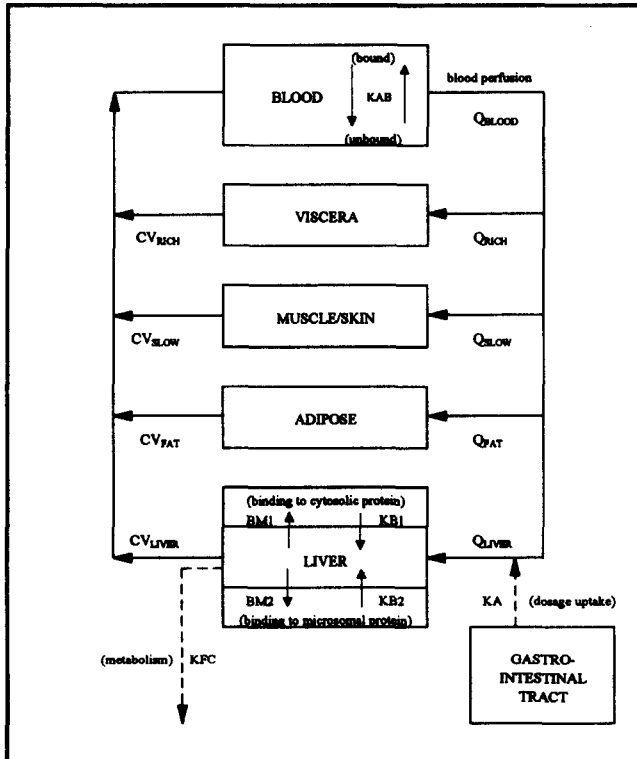


Figure 2. Diagram of the Physiologically-Based Pharmacokinetic Model for Rodents

Table 2. Model Parameters for the Rodent PB-PK Model (Leung et al., 1988; Leung et al., 1990a)

PARAMETER	Abbreviation	Units	Rat	C57 Mouse	DBA Mouse	B6 Mouse
BODY WEIGHT	BW	(kg)	0.3	0.0231	0.0239	0.0243
COMPARTMENT VOLUMES						
Liver	VLIVER	(m ³)	1.50E-05	1.16E-06	1.20E-06	1.22E-06
Richly Perfused	VRICH	(m ³)	1.20E-05	9.24E-07	9.56E-07	9.72E-07
Slowly Perfused	VSLOW	(m ³)	2.13E-04	1.76E-05	1.68E-05	1.87E-05
Fat	VFAT	(m ³)	3.30E-05	1.36E-06	2.74E-06	1.22E-06
Blood	VBLOOD	(m ³)	1.50E-05	1.16E-06	1.20E-06	1.22E-06
BLOOD FLOW						
Liver	QLIVER	(m ³ /h)	1.44E-03	2.15E-04	2.21E-04	2.21E-04
Richly Perfused	QRICH	(m ³ /h)	2.93E-03	4.39E-04	4.50E-04	4.50E-04
Slowly Perfused	QSLow	(m ³ /h)	1.09E-03	1.46E-04	1.50E-04	1.50E-04
Fat	QFAT	(m ³ /h)	2.87E-04	6.03E-05	6.18E-05	6.18E-05
Cardiac Output	QBLOOD	(m ³ /h)	5.74E-03	8.61E-04	8.83E-04	8.83E-04
PARTITION COEFFICIENTS (Tissue:Blood Ratio)						
Liver	RLIVER	(unitless)	20	20	20	20
Richly Perfused	RRICH	(unitless)	20	20	20	20
Slowly Perfused	RSLOW	(unitless)	40	250	250	250
Fat	RFAT	(unitless)	350	350	350	350
BIOCHEMICAL CONSTANTS						
Binding capacity to cytosolic protein	BM1	(mol)	5.40E-11	4.20E-12	4.20E-12	4.20E-12
Binding dissociation constant to cytosolic protein	KB1	(mol/m ³)	1.50E-08	2.90E-07	2.00E-06	2.90E-07
Binding capacity to microsomal protein	BM2	(mol)	-	2.00E-08	2.00E-08	2.00E-08
Non-induced binding capacity to microsomal protein	BM2non	(mol)	1.00E-08	-	-	-
Induced binding capacity to microsomal protein	BM2ind	(mol)	8.50E-08	-	-	-
Binding dissociation constant to microsomal protein	KB2	(mol/m ³)	7.00E-06	2.00E-05	7.50E-05	2.00E-05
First-order metabolic rate constant	KFC	(1/hour)	2	3.25	1.75	3.25
Absorption rate constant	KA	(1/hour)	0.2	0.02	0.02	0.02
Blood binding constant	KAB	(unitless)	2.5	2.5	2.5	2.5

in the human model since at the relatively low background TCDD concentrations to which humans are exposed, the concentrations of TCDD are expected to be insufficient to cause induction. The incorporation of protein induction processes in the rodent model is important, not only because liver binding proteins increase the amount of TCDD in the liver, but also because different species have different activities and binding affinities for TCDD, causing interspecies variability in tissue bioaccumulation and internal distribution.

Model Verification:

To assess the predictive ability of the rodent models, we conducted model simulations in which the rodent species were exposed to TCDD at doses similar to those used in actual bioassays (Rose et al., 1976; Gasiewicz, 1983). The model-predicted concentrations of TCDD were then compared to observed concentrations in these studies. To test the human model, we performed a computer simulation in which a 70 kilogram reference human is exposed to “background” levels of TCDD through the consumption of TCDD contaminated food. A literature compilation of available estimates of background TCDD intake of the general population in industrialized nations (Table 3) provided estimates ranging from 12.0 to 34.8 picograms per day, with a mean of 22.1 picograms per day, or 0.32 pg/kg/day for a reference human of 70 kg. The relatively small variability in these estimates, in spite of the differences in sampling locations and study designs, suggests that dioxin exposure in the general population is relatively constant across geographic regions. We then compared the results of the model-calculated TCDD concentrations in the liver and adipose tissue to observations from actual liver and adipose tissue specimens collected from autopsy patients. Fifteen separate estimates of mean TCDD concentrations in adipose tissue were obtained from the literature (Figure 3). These estimates represent subjects from Canada, U.S., Europe, and Japan, who have experienced no known exposure to elevated TCDD levels. The mean concentrations of TCDD in adipose tissue range from a minimum of 5.4 pg/g to a maximum of 11 pg/g. The estimates do not display any geographical pattern or skewness, suggesting that background exposure to TCDD is reasonably constant across industrialized nations. The mean of all estimates of TCDD concentration in the adipose tissue is 8.0 pg/g, and the standard deviation is 1.8. The mean concentration is 7.5 pg/g once the estimates are weighted by the number of tissue samples analyzed in each study. Liver concentration data (n=26) from Leung (1990c) exhibit a geometric mean liver concentration of TCDD of 0.70 pg/g. Since the majority of the autopsy cases represent middle-aged or elderly adults, most observations represent near steady-state conditions.

Model Simulations:

To investigate the relationship between the external dose of TCDD and resulting internal concentrations of TCDD in the liver, adipose tissue and other organs of humans, B6 mice, and Sprague-Dawley and Osborne-Mendel rats, we conducted model simulations for the following TCDD dosing scenarios:

<u>B6 Mouse:</u>	1390, 5550, 6940, 27800, 69400, 278000 pg/kg/day
<u>Rat:</u>	35, 117, 1000, 1170, 1390, 6940, 10000, 11700, 51200, 69400, 100000, 248000 pg/kg/day
<u>Human:</u>	0.32 pg/kg/day

Table 3. Estimates of Human Background Exposure to TCDD from Several Studies

pg/day	pg/kg/day	Source	Country and Method
12.0	0.17	EPA (1994)	North America (EPA reassessment)
15.9	0.23	Henry et al. (1992)	U.S. (Food and Drug Administration approach)
17.4	0.25	Ono et al. (1987)	Japan (Market basket estimates)
20.0	0.29	Theelen (1991)	Netherlands (Institute of Public Health)
25.0	0.36	Furst et al. (1991)	Germany (Analysis of food samples)
25.2	0.36	Beck et al. (1994)	Germany (Market basket estimates)
26.7	0.38	Ontario Min. of Env. (1988)	Canada (Market basket estimates)
34.8	0.50	Travis and Hattemer-Frey (1991)	U.S. (Fugacity food chain model predictions)
22.1	0.32	Several	Average of All Above Estimates.

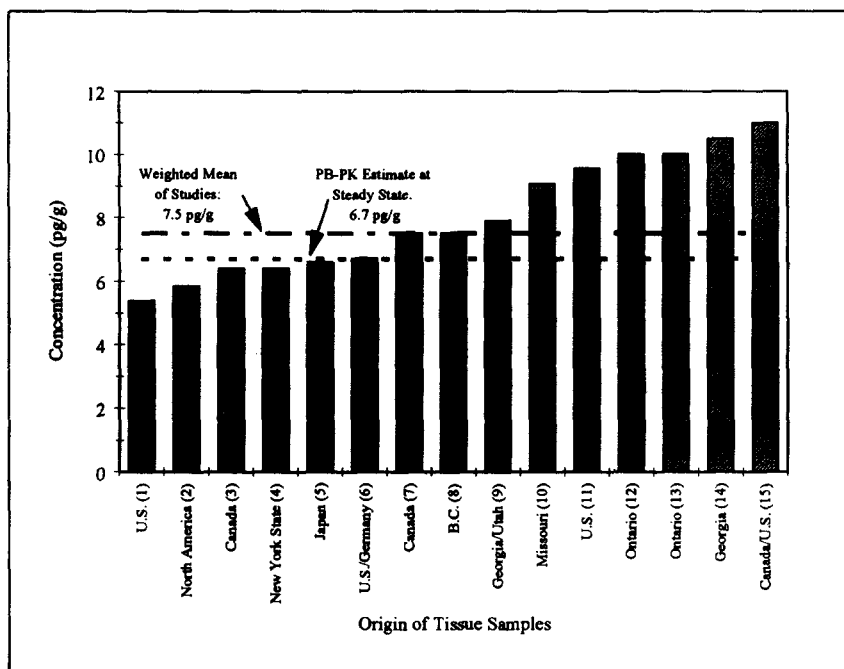


Figure 3. Measured adipose tissue concentrations of TCDD in the general population, compared with steady state adipose concentration of TCDD produced by the pharmacokinetic model, assuming a background exposure of 0.32 pg/kg/day. Data sources are: ⁽¹⁾ and ⁽²⁾ EPA (1994b); ⁽³⁾ and ⁽⁵⁾ Ryan (1986); ⁽⁴⁾ ⁽⁸⁾ ⁽¹³⁾ Ryan et al. (1985); ⁽⁶⁾ Schechter (1991); ⁽⁷⁾ Graham et al. (1984); ⁽⁹⁾ Patterson et al. (1986); ⁽¹⁰⁾ Leung et al. (1990); ⁽¹¹⁾ Needham et al. (1987); ⁽¹²⁾ Ryan (1984); ⁽¹⁴⁾ Patterson et al. (1994); ⁽¹⁵⁾ Ryan and Williams (1983).

The dosing scenarios for the rats and mice were chosen to mimic actual experimental bioassays, as identified in a database of chronic cancer potency experiments (Gold et al., 1984, 1986, 1991, 1993). The dosing scenario for humans was chosen to reflect actual background exposure. The rationale for the dosing scenarios is twofold. First, the model simulations are conducted within the range of dosages for which the physiological models were developed. Second, the exposure levels reflect conditions under which rodent-to-human extrapolations are made in risk assessments.

The relationship between the external dose and the steady-state concentration of TCDD is expressed as a simple ratio B , which can be referred to as a "bioaccumulation efficiency" as it expresses the magnitude of the concentration in tissue and target organs resulting from a certain "external dose":

$$B_{\text{liver}} = \frac{\text{TCDD concentration in the liver (pg / g)}}{\text{External dose per unit body weight (pg / kg / day)}}$$

A similar ratio was developed by Scheuplein and Bowers (1995) and proved to be useful in the data interpretation. Bioaccumulation efficiencies were investigated for all tissues and organs in the model, but in this paper we limit the discussion to those for the liver B_{liver} (i.e. $C_{\text{liver}}/\text{external dose}$) and the adipose tissue B_{adipose} (i.e. $C_{\text{adipose}}/\text{external dose}$). Liver concentrations are of particular interest since the liver is the primary site for the incidence of TCDD induced cancerous tumors in several animal species, and liver tumor incidence data in rodents form the basis of nearly all traditional TCDD cancer risk estimates, including those by U.S. and Canadian federal agencies.

RESULTS

Model Verification

Figure 4 illustrates the time course of the disposition of TCDD in the various human body tissues for a 70 year simulation based on background exposure of 0.32 pg/kg/day. Model simulations reveal that TCDD is absorbed slowly over time with tissue concentrations approaching 95% of steady-state levels after approximately 40 years. The reason for this slow time response is the large capacity of the fatty tissue to retain TCDD, and the negligible metabolic transformation of TCDD. The adipose tissue compartment in large part drives the overall model response and is the primary reservoir for storage of TCDD in the human body (Figure 4). The steady-state concentration of TCDD in adipose tissue predicted by the model based on the background exposure of 0.32 pg/kg/day, is approximately 6.7 pg/g (Figure 3). The differences in the steady-state concentrations of TCDD between the compartments are proportional to the tissue:blood partition coefficients in the model. The corresponding steady-state concentration of TCDD in the liver is 0.56 pg/g.

Figure 3 illustrates that the model predicted concentration in human adipose tissue of 6.7 pg/g is in good agreement (i.e. the weighted mean of all observed concentrations is 7.5 pg/g) with observed concentrations, but also that observed mean concentrations differ by approximately a factor of 2, (i.e. from 5.5 to 11 pg/g), due to

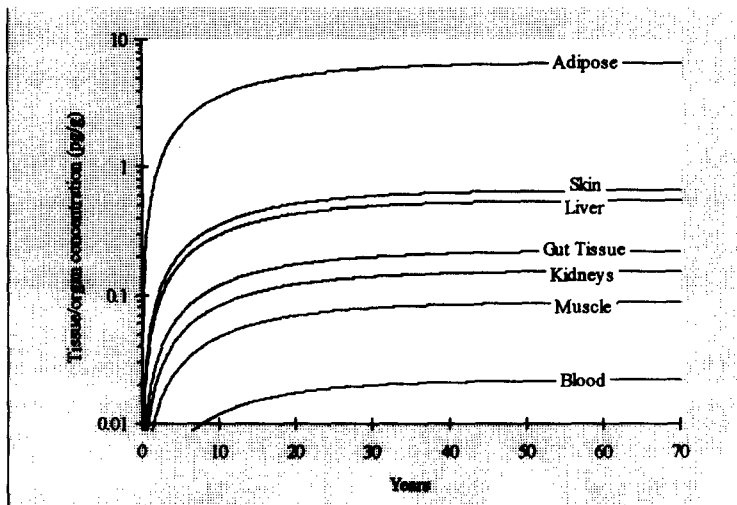


Figure 4. Disposition of TCDD in human tissues and organs assuming a daily background exposure of 0.32 pg/kg/day, as predicted by the pharmacokinetic model.

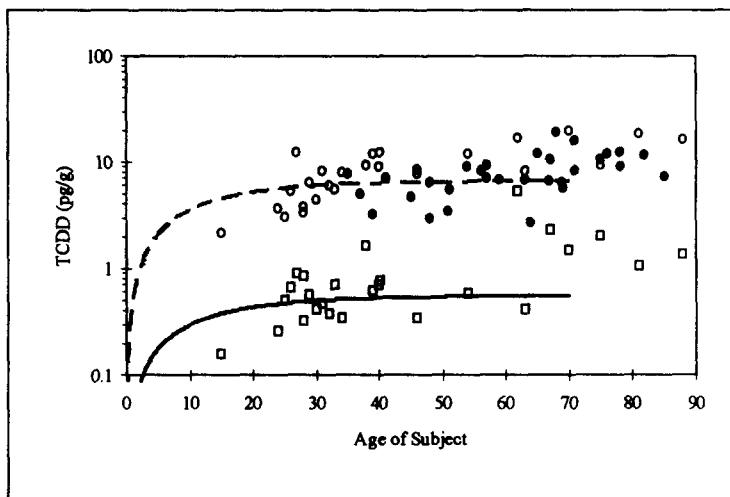


Figure 5. Comparison of measured TCDD concentrations in adipose tissue samples (circles) and liver samples (squares) obtained from human autopsy studies, to pharmacokinetic model predictions assuming constant background exposure of 0.32 pg/kg/day for a 70 kg human. Sources: Leung et al. (1990c) [open symbols]; Patterson et al. (1986) [filled symbols].

sampling variability. The geometric mean of the observed liver concentrations of 0.70 pg/g is in good agreement with the model predicted concentration of 0.56 pg/g, and the ratio between liver and adipose tissue TCDD concentrations is similar between the empirical results and the model (approximately 1:10). When data from the model and the empirical studies are expressed on a total lipid basis, liver concentrations are very similar to adipose tissue concentrations, indicating that the human liver accumulates TCDD principally on the basis of tissue solubility (Leung et al., 1990a) at background exposure levels.

Figure 5 compares the model simulation results over time to observed data from two autopsy studies (Leung et al., 1990c; Patterson et al., 1986). The comparison is limited to these two studies since the other data sources in Figure 3 did not indicate the ages of the subjects. Figure 5 illustrates that the observed concentrations of TCDD compare favorably with the model predictions, although there is a tendency for the model to somewhat underpredict TCDD concentrations in people older than 60 years. This apparent discrepancy increases with the age of the autopsy patients. However, it should be noted that the Leung et al. (1990) study produced tissue concentrations which were higher than most other autopsy assessments (see Figure 3), so the degree of underestimation is not as great as is suggested in Figure 5. Furthermore, it must be recognized that there is some uncertainty (Table 3) in the background exposure estimate and its change over time.

While the human pharmacokinetic model produced predicted tissue TCDD concentrations that were very similar to averaged results from many autopsy-based studies, there is some discrepancy between observed and predicted TCDD concentration time trends. Actual tissue specimens indicate that TCDD concentrations increase with age over an entire lifetime, while model simulation concentrations do not increase significantly past approximately 50 years. The simplified parameterization of the PB-PK model may in large part be responsible for this difference, as the model assumes constant body size and composition over the entire 70-year simulation. On average, adults tend to increase in weight with age, and the percentage of body fat also increases. Both of these factors would contribute to higher concentrations in the elderly than those predicted by the model for a simplified reference human. It is believed that the performance of the model could therefore be improved through the incorporation of realistic changes in body type over a simulated lifetime. Despite this problem, the rate of accumulation of TCDD indicated by the model is consistent with independent estimates of the half-life of elimination of TCDD. Some published estimates of the half-life of TCDD in humans are 7.5 years (Aylward et al., 1996), 6 to 9 years (Scheuplein and Bowers, 1995), and 5 to 10 years (Poiger and Schlatter, 1986). Given that the model is highly simplified and that the background exposure estimate contains considerable uncertainty (Table 3), the model fits the independently measured human tissue data remarkably well. Cumulatively, these findings provide confirmation of the effectiveness of pharmacokinetic models in describing the dispositional behavior of TCDD in humans at background exposure levels.

Figure 6 illustrates the rodent model's ability to predict the bioaccumulation and internal distribution of TCDD, by comparing the model results to those from laboratory experiments in which the time course and tissue distribution of TCDD in rodents were documented (Rose et al., 1976; Gasiewicz, 1983). Examples of model simulations for B6 mice and Sprague Dawley rats are presented in Figure 6. Other simulations (not shown) were performed for other rodent strains and dosing regimes. As further supported by Leung et al. (1988, 1990a), the rodent PB-PK model does an adequate job of describing the relationship between the external dose and liver and adipose tissue concentrations in rodent species. The quality of fit is somewhat better for the rat than for the mouse, possibly due to the lack of a dose-dependent hepatic protein binding mechanism in the mouse model. Model simulations for rodents indicate that in response to chronic dosing of TCDD, the rodent tissue compartments respond rapidly in both rats and mice compared to humans. Sprague Dawley rats approached steady-state

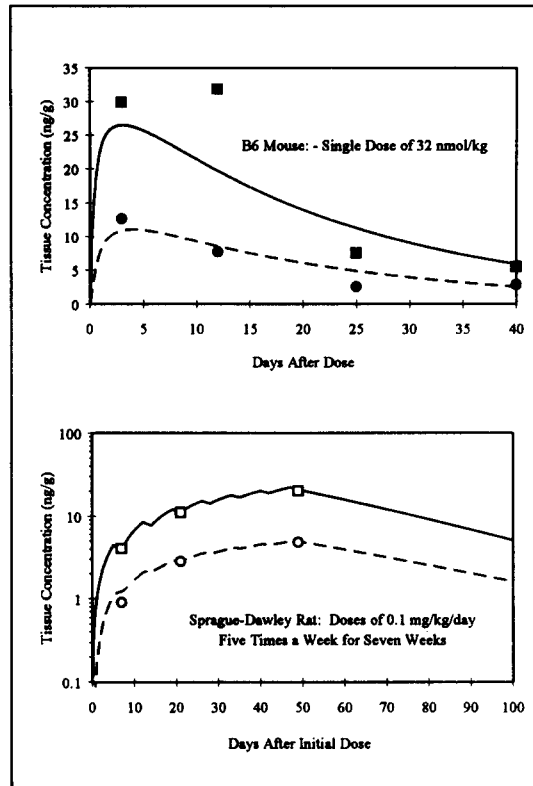


Figure 6: Comparison of predicted vs. observed TCDD tissue concentrations in experimental animals. Lines represent PB-PK model simulations in adipose tissue (broken) and the liver (solid). Open symbols represent data from Rose et al. (1976); filled symbols represent data from Gasiewicz (1983).

conditions after 3 to 5 months of continuous exposure, equivalent to a half-life of elimination in adipose tissue of approximately one month. The rate of TCDD accumulation in adipose tissue for the mouse strains (C57, B6, DBA) in the model simulations was more rapid, with steady-state conditions approached after approximately two weeks of continuous exposure.

Model Simulation of Human - Rodent Extrapolation

A summary of the results of model simulations that were conducted to reconstruct the pharmacokinetics of TCDD in rodents used in the bioassays that are the basis for TCDD risk assessment is presented in Table 4. Considering that the models were earlier shown to be in reasonable agreement with available data sets, the model results are expected to give a realistic representation of the response of the TCDD concentrations in the various tissues and organs of humans and the two rodent species to different dose levels. Table 4 illustrates that the bioaccumulation efficiencies for the liver B_{liver} , i.e. the ratio of the steady-state liver concentration and the external

Table 4. Bioaccumulation Efficiencies, Interspecies Extrapolation Factors, Times Required to Achieve 95% of Steady-State and Approximate Elimination Rate Constants of TCDD in Mice, Rats, and Humans

	Mouse	Rat	Rat	Rat	Human
Dose Range Simulated (pg/kg/day):	(1,390-278,000)	(35-1,390)	(6,940-11,700)	(51,200-248,000)	(0.32)
Adipose Bioaccumulation Efficiency ($B_{adipose}$)	0.031	0.10	0.10	0.10	22
Liver Bioaccumulation Efficiency (B_{liver})	0.072	0.16	0.29	0.48	2.2
PB-PK Interspecies Extrapolation Factor (Adipose)	725	215	214	214	1.0
PB-PK Interspecies Extrapolation Factor (Liver)	31	14	7.8	4.6	1.0
Interspecies extrapolation factor based on mg/kg/day	1.0	1.0	1.0	1.0	1.0
Interspecies extrapolation factor based on mg/kg^{0.75}/day	410	60	60	60	1.0
Time to 95% Steady State (t_{95}) in Adipose tissue (days)	69	89	110	140	14000
Approximate Rate Constant ($k = 3/t_{95}$) (1/day)	0.043	0.034	0.027	0.021	0.00021

dose, range between 0.07 for the mouse to 2.2 for humans. The results indicate that, given the same continuing external dose, TCDD concentrations human livers achieve much greater concentrations than corresponding concentrations in rats and mice. Given an equivalent external dose, steady-state concentrations of TCDD in human livers are 31 times greater than those in mice and 4.6 to 14 times greater than those in rats, depending on the TCDD dosage used in the rat bioassay. The B_{liver} values increase with the dose level in rats, hence becoming closer to that of humans, as a result of the induction of TCDD binding hepatic proteins at these very high dose levels. The bioaccumulation efficiencies in the adipose tissue, $B_{adipose}$, which represent the steady-state TCDD concentration in the adipose tissue as a result of the external dose administered, vary among humans and mice by 725 fold and among humans and rats by 214 fold, indicating that TCDD concentrations in the adipose tissue of humans can reach values that are orders of magnitude greater than those in mice and rats when a similar external dose is applied. In contrast to B_{liver} , $B_{adipose}$ is not dependent of the dose administered because of the absence of inducible TCDD proteins in the adipose tissue. It can be argued that humans and rodents are typically not exposed to the same dose levels, as most toxicity experiments require very high dose levels to measure a statistically significant effect. However, in a typical risk assessment, the results from the test animals conducted at the high dose levels are extrapolated to the low doses to which humans are exposed under the assumption that humans respond in a "similar" manner to the chemical as the test animals as long as the dose is scaled to body-weight. As a result, the B values for rodents are applied to humans in a risk assessment. When this is done, Table 4 illustrates that bioassays in rodents will underestimate the internal target organ concentration by a very large amount due to differences in pharmacokinetics alone. The interspecies extrapolation factors, representing the ratio of bioaccumulation efficiencies in humans and rodents, reflect this level of underestimation. Scaling the external dose to body weight to the power 0.75, as proposed by the U.S. EPA and FDA, reduces this level of underestimation considerably. However, Table 4 illustrates that the level of underestimation of the adipose tissue concentration is still substantial, whereas this method of interspecies scaling will result in a 4 to 13 fold overestimation of dioxin concentrations in human liver tissue.

DISCUSSION

This study shows that the relationship between the external dose and tissue concentrations for TCDD can differ between humans and rodents by orders of magnitude. This indicates that the underlying assumption of similarity in dosimetry between rodents and humans is incorrect and tends to greatly underestimate potential cancer risks if the second assumption of equal sensitivity between rodents and humans holds. If a cross-species scaling factor expressing body weight to the power of 0.75 is used, major discrepancies in the relationships between external dose and tissue concentrations of TCDD remain and can lead to a substantial underestimation as well as overestimation of potential cancer risks.

The results also indicate that in a typical non-cancer risk assessment for TCDD, where a safety or uncertainty factor of 1 to 10 has often been used for interspecies extrapolation, the safety factor is too small to account for pharmacokinetically controlled differences between humans and rodents alone. When extrapolating

TCDD related effects observed in rodents to humans based on the external dose, assuming the liver is the most likely target organ, a safety factor of 31 is suggested for dosimetry related mouse-to-human extrapolation, and a factor of 4.6 to 14, depending on the dosage used in the bioassay, is suggested for dosimetry related rat-to-human extrapolation. If the adipose tissue is the site of action, these dosimetry related safety factors in mice and rats are 726 and 214 respectively. These factors do not account for differences in interspecies sensitivity to dioxin (discussed below) which must also be taken into consideration. If TCDD is found to be less potent in humans than rodents, as has been postulated by many scientists, smaller safety factors than those described above would be required.

The main reason that simple body-weight scale up methods fail to correctly represent the relationship between external dose and internal tissue concentration is that the fraction of the total TCDD body burden that is eliminated per unit of time, i.e. the elimination rate constant k , drops with increasing body weight of the organism. Since the external dose, expressed in units of mg chemical per kg of organism body weight per day, remains constant with increasing body weight, the internal concentrations increase with increasing body weight, causing steady-state concentrations in larger organisms to reach much greater values than those in smaller organism when given the same external dose. Similar body weight depending relationships for the elimination of hydrophobic substances have been observed in other studies (Walker, 1978; Gobas and Mackay, 1987), and ultimately relate to the drop in area/volume ratio with increasing volume. This principle can be easily demonstrated in a simple two-compartment model where the concentration in the organism is C_o (g/kg), the chemical is administered in a dose D (g/day), V_o is the weight of the organism in kg, and the chemical is eliminated at a rate constant k with units of days⁻¹ and t is time in days. The differential equation for this model is:

$$\frac{d(V_o \cdot C_o)}{dt} = D - (k \cdot V_o \cdot C_o)$$

Dividing both sides by V_o gives a steady-state solution (i.e. $dC_o/dt = 0$) in which C_o equals the external dose D^* , i.e. D/V_o , divided by k , i.e. C_o equals D^*/k . Since the elimination rate constant of TCDD in mammals drops with increasing body weight (Table 4; Walker, 1978), C_o will increase with increasing body weight since the external dose D^* is constant with increasing body weight. The result of this is that at the same external dose, the internal concentration C_o will increase with increasing body weight (Figure 7). This effect is not specific to TCDD, but applies to many substance as long as the elimination process involves a passive transport process. While the simplified model above helps to explain observed differences in bioaccumulation efficiencies among species, there are other pharmacokinetic factors which are also important, and which are accounted for in the models used in this study. For example, species specific differences in metabolic transformation rates of a chemical can alter the relationship between weight and internal concentration under a scenario of administering the same external dose. Also, differences in the chemical storage capacities of individual target organs among organisms (e.g. due to size and lipid content) can have an important effect on the chemical's bioaccumulation efficiencies for the various

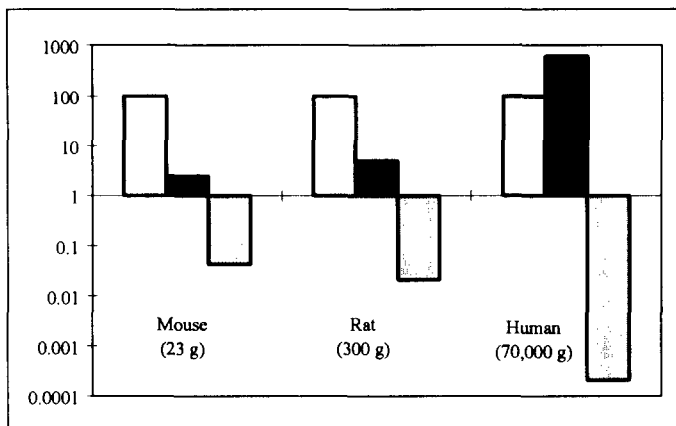


Figure 7. Illustrative example of the relationship between the external dose (white, in units of pg/kg/day, and set at an illustrative value of 100 pg/kg/day), the elimination rate constant (gray, in units of d⁻¹, data from Table 4), the internal concentration in the organism (black, in units of ng/kg), and the organism's body weight (in units of grams, data from Table 3).

organs. It is recommended that simple whole-organism-body-weight scaling be abandoned in risk assessments of TCDD and other compounds. The internal tissue concentrations in relevant organs (e.g. liver in case of TCDD) provide a better surrogate for the effective dose on which the risk assessment can be based. This has been suggested before (e.g. Rozman et al., 1993; Bull et al., 1993), but this study stresses the need to do this as simple body-weight-scale-up methods are shown to lead to large errors in the estimation of the effective dose of TCDD in relevant target tissues. Against the use of internal tissue concentrations it can be argued that “the conservatism in animal based risk assessments originates from the current procedures used in high-to-low dose extrapolations, not from any supposed inherent differences in species sensitivity” (Scheuplein and Bowers, 1995). However, it should be realized that when body-weight-scale-up methods remain in use, the level of conservatism is substantially lower than is believed. The use of internal tissue concentrations as a surrogate for the effective dose in cancer risk assessments implies that internal concentrations of the chemical should be measured in addition to the external dose as part of bioassays. In contrast to an earlier era where such measurements could not be made, accurate measurements of internal tissue concentrations of TCDD and other compounds are increasingly possible due to advances in environmental chemistry. Where such measurements cannot be made, pharmacokinetic models provide a reliable alternative for estimating internal tissue concentrations from the external dose. Although these models contain a certain amount of uncertainty (Edler and Portier, 1992), i.e. approximately a factor of 2 for TCDD in our studies, this uncertainty is small compared to the error made in the risk assessment when the external dose is selected as the surrogate for the effective dose. The results from pharmacokinetic models regarding the relationship between the external dose and relevant internal tissue concentrations can be expressed in terms of interspecies extrapolation factors as is done in Table 4. These factors can be used to “translate” the external dose-risk relationship observed in the test organism (e.g. rodent) to those in humans or they can be simply used as one of

several "safety factors" in a hazard assessment. The use of internal concentrations over the external dose as the chief surrogate for risk assessments does not directly address the problem of high to low dose extrapolation in risk assessments. However, better characterization of the effective dose in animal studies through internal tissue concentration measurements or pharmacokinetic modelling is likely to enhance insights into the relationship between dose and effect, and contribute to improved risk assessment.

IMPLICATIONS FOR TCDD RISK ASSESSMENT

Traditional TCDD risk assessment approaches, which use the external dose as the basis for interspecies extrapolation, have a weak scientific basis when compared to the use of surrogate measures for the target specific exposure, e.g. liver or lipid concentrations. The application of pharmacokinetic principles, along with the selection of an appropriate surrogate for target dose, bring a degree of biological realism to risk assessment, and help to narrow the knowledge gap between gross external exposure to chemicals and the toxic responses of interest (Edler and Portier, 1992). While additional knowledge is required to extend TCDD risk assessment to the molecular level, new information can be incorporated into risk assessments while still acknowledging the importance of dosimetry.

The relationship between external dose and cancer risk involves two major components, i.e. "dosimetry", which determines the concentrations in the internal tissues that are reached given a certain external dose, and "sensitivity", controlling the extent of effect (e.g. tumor formation for TCDD) at the target tissue concentration. First, we must address interspecies differences in the physiological processes which translate the administered external dose of chemical to an effective target tissue dose. Terms used to describe these processes include pharmacokinetics, dosimetry, toxicokinetics, allometric variation, and biotransformation. This paper illustrates the magnitude of these interspecies differences for TCDD, and it is shown that these differences can be estimated using simple physiologically based models. Second, we must evaluate interspecies differences in the ability of those biologically relevant doses, or "effective doses", to elicit adverse responses such as cancer. These are typically referred to as interspecies differences in chemical "sensitivity", "susceptibility", or "pharmacodynamics". This component of interspecies extrapolation is less well understood. A major problem in the assessment of relative sensitivities of humans and rodents is that the rodent bioassays for TCDD and human studies suggest different target tissues/organs for the carcinogenic action of TCDD. Bioassays indicate significant increases in the incidence of rodent hepatocarcinomas, while epidemiological studies (although limited in their ability to detect significant effects) have demonstrated little or no evidence for a similar response in the human liver, but do indicate significant increases in total cancers and particularly cancers of the respiratory tract (Fingerhut et al., 1991).

Recently, a clearer picture has emerged regarding the relative contributions of "pharmacokinetics" and "pharmacodynamics" in dioxin risk assessment. Although the recent reassessment of dioxin by the U.S. Environmental Protection Agency concluded that humans and experimental animals can be reasonably assumed to be of equal sensitivity for many health endpoints (U.S. EPA, 1994a), there is a growing body of evidence

suggesting that humans may not be as sensitive to dioxin as rodents. Mechanistic models (e.g. Kohn et al., 1995) have been developed to investigate the importance of biochemistry in relation to tumor formation. Aylward et al. (1996) reexamined the most significant epidemiology study for dioxin, that of the National Institute of Safety and Health (Fingerhut et al., 1991). They concluded that once rats and humans are scaled to a biologically relevant dose (i.e. TCDD concentration in serum lipids), humans appear to be considerably less susceptible to the carcinogenic effects of TCDD when compared to rats. When peak or average serum lipid concentration is used as a dosimetric, human cancer responses are 4 to 9-fold lower than those in rats, and when serum lipid area-under-the-curve is used as a dosimetric, rodents are determined to be up to two orders of magnitude more sensitive to cancer effects than humans. This emerging information on the relative sensitivities of rodents and humans to equal biologically relevant doses of dioxin can be combined with the pharmacokinetic principles discussed in this paper to produce more realistic estimates of human cancer risk from TCDD exposure. The interspecies extrapolation factors in Table 4, which specifically relate to differences in toxicokinetics between different species, can be used together with newly developed factors that relate tissue sensitivities between species to more reliably predict effects in humans in response to TCDD intakes from those observed in test organisms. Previous approaches, in which interspecies extrapolation of cancer risks (i.e. dosimetry and sensitivity) involved simple body-weight or surface area scaling, should be replaced by a method which uses two types of extrapolation factors, one to account for pharmacokinetic factors, and the other to account for sensitivity differences. In this manner, as more information becomes available on the relative tissue-responses of rodents and humans, it can be incorporated into a more biologically-based approach to risk assessment. PBPK models as well as other pharmacokinetic models (e.g. Carrier et al. 1995, Van der Molen et al. 1996) can play a useful role in quantifying the pharmacokinetic differences between different types of species of organisms and humans.

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APPENDIX A - Pharmacokinetic Model for Humans

In humans, the tissue distribution of TCDD is determined primarily by the intrinsic partitioning properties of the various tissues, and flows between compartments are accurately described by fugacity-based partitioning behaviour:

$$C_i = Z_i * f_i$$

- C_i = concentration of TCDD in compartment i (mol/m³)
 Z_i = fugacity capacity of compartment i (mol/m³Pa)
 f_i = fugacity (escaping tendency) of TCDD in compartment i (Pa)

Fat, Skin, Muscle and Richly Perfused Compartments

$$df_i/dt = (Q_i * Z_{\text{BLOOD}} * (f_{\text{BLOOD}} - f_i)) / (V_i * Z_i)$$

- i = tissue compartment i (= fat, skin, muscle, richly perfused tissue)
 Q_i = blood perfusion through compartment i (m³/hour)
 Z_{BLOOD} = fugacity capacity of arterial blood entering compartment (mol/m³Pa)
 f_{BLOOD} = fugacity of arterial blood entering compartment (Pa)
 V_i = volume of compartment i (m³)

Blood Compartment

$$df_{\text{BLOOD}}/dt = [(Q_{\text{FAT}} * f_{\text{FAT}}) + (Q_{\text{SKIN}} * f_{\text{SKIN}}) + (Q_{\text{MUSCLE}} * f_{\text{MUSCLE}}) + (Q_{\text{RICH}} * f_{\text{RICH}}) + ((Q_{\text{LIVER}} + G_{\text{URINE}}) * f_{\text{LIVER}}) + ((Q_{\text{KIDNEYS}} - G_{\text{URINE}}) * f_{\text{KIDNEYS}}) - (Q_{\text{BLOOD}} * f_{\text{BLOOD}})] / V_{\text{BLOOD}}$$

- Q_{FAT} = blood perfusion through fat compartment (m³/hour)
 Q_{SKIN} = blood perfusion through skin compartment (m³/hour)
 Q_{MUSCLE} = blood perfusion through muscle compartment (m³/hour)
 Q_{RICH} = blood perfusion through richly perfused tissue compartment (m³/hour)
 Q_{LIVER} = blood perfusion through liver (m³/hour)
 Q_{KIDNEYS} = blood perfusion through kidneys (m³/hour)
 Q_{BLOOD} = cardiac output (m³/hour)
 G_{URINE} = urine flow rate (m³/hour)
 V_{BLOOD} = volume of blood compartment (m³)

Liver Compartment

$$df_{\text{LIVER}}/dt = (Q_{\text{LIVER}} * Z_{\text{BLOOD}} * (f_{\text{BLOOD}} - f_{\text{LIVER}}) - (k_M * Z_{\text{LIVER}} * V_{\text{LIVER}} * f_{\text{LIVER}})) / (V_{\text{LIVER}} * Z_{\text{LIVER}})$$

- k_M = metabolic transformation rate constant (1/hour)

$$Z_{LIVER} = \text{fugacity capacity of the liver (mol/m}^3\text{Pa)}$$

$$V_{LIVER} = \text{volume of liver (m}^3\text{)}$$

Kidneys

$$df_{KIDNEYS}/dt = [(Q_{KIDNEYS} * Z_{BLOOD} * f_{BLOOD}) - (G_{URINE} * Z_{URINE} * f_{KIDNEYS}) - ((Q_{KIDNEYS} - G_{URINE}) * Z_{BLOOD} * f_{KIDNEYS})] / (Z_{KIDNEYS} * V_{KIDNEYS})$$

$$Q_{KIDNEYS} = \text{blood perfusion through kidneys (m}^3\text{/hour)}$$

$$V_{KIDNEYS} = \text{volume of kidneys (m}^3\text{)}$$

$$Z_{KIDNEYS} = \text{fugacity capacity of kidneys (mol/m}^3\text{Pa)}$$

$$Z_{URINE} = \text{fugacity capacity of urine (mol/m}^3\text{Pa)}$$

Gut Lumen Compartment

$$df_{GUTLUMEN}/dt = [(G_{DIET} * Z_{DIET} * f_{DIET}) + (G_{BILE} * Z_{BILE} * f_{LIVER}) - (G_{FECES} * Z_{GUTLUMEN} * f_{GUTLUMEN}) + (D_{GUT} * (f_{GUTTISSUE} - f_{GUTLUMEN}))] / (Z_{GUTLUMEN} * V_{GUTLUMEN})$$

$$D_{GUT} = (G_{FECES} * Z_{GUTLUMEN} * \epsilon) / (1 - \epsilon)$$

$$G_{FECES} = 0.35 * G_{DIET}$$

$$Z_{GUTLUMEN} = 0.40 * Z_{DIET}$$

$$G_{DIET} = \text{food consumption rate (m}^3\text{/hour)}$$

$$Z_{DIET} = \text{fugacity capacity of diet (mol/m}^3\text{Pa)}$$

$$f_{DIET} = \text{fugacity in diet (Pa)}$$

$$G_{BILE} = \text{flow rate of bile (m}^3\text{/hour)}$$

$$Z_{BILE} = \text{fugacity capacity of bile (mol/m}^3\text{Pa)}$$

$$G_{FECES} = \text{fecal excretion rate (m}^3\text{/hour)}$$

$$Z_{GUTLUMEN} = \text{fugacity capacity of gut lumen (mol/m}^3\text{Pa)}$$

$$\epsilon = \text{dietary uptake efficiency (unitless)}$$

$$D_{GUT} = \text{diffusion rate across gut (mol/hourPa)}$$

$$V_{GUTLUMEN} = \text{volume of gut lumen (m}^3\text{)}$$

Gut Tissue Compartment

$$df_{GUTTISSUE}/dt = [(Q_{GUTTISSUE} * Z_{BLOOD} * f_{BLOOD}) - ((Q_{GUTTISSUE} + G_{BILE} + G_{URINE}) * Z_{BLOOD} * f_{GUTTISSUE}) + (D_{GUT} * (f_{GUTLUMEN} - f_{GUTTISSUE}))] / (Z_{GUTTISSUE} * V_{GUTTISSUE})$$

$$Q_{GUTTISSUE} = \text{blood perfusion through gut tissue (m}^3\text{/hour)}$$

$$V_{GUTTISSUE} = \text{volume of gut tissue (m}^3\text{)}$$

$$Z_{GUTTISSUE} = \text{fugacity capacity of gut tissue}$$

APPENDIX B - Pharmacokinetic Model for Rodents

Liver Compartment

$$dA_{LIVER}/dt = Q_{LIVER} * (CA - CV_{LIVER}) - dAM/dt + [(dAP/dt) / (1 + KAB)]$$

$$dAP/dt = KA * AP$$

$$AP = \text{Dose} * e^{-KA * t}$$

$$dAM/dt = KFC / (BW)^{0.3} * CV_{LIVER} * V_{LIVER}$$

$$C_{LIVER} = A_{LIVER} / V_{LIVER}$$

$$A_{LIVER} = (V_{LIVER} * CV_{LIVER} * R_{LIVER}) + [(BM1 * CV_{LIVER}) / (KB1 + CV_{LIVER})] + [(BM2 * CV_{LIVER}) / (KB2 + CV_{LIVER})]$$

$$CV_{LIVER} = A_{LIVER} / [(V_{LIVER} * R_{LIVER}) + (BM1 / (KB1 + CV_{LIVER})) + (BM2 / (KB2 + CV_{LIVER}))]$$

$$BM2 \text{ (total)} = BM2_{non} + [(CV_{LIVER} * BM2_{ind}) / (KB1 + CV_{LIVER})]$$

A_{LIVER} = TCDD in liver tissue (mol)

Q_{LIVER} = blood perfusion (flow) through liver compartment (m^3 /hour)

CA = concentration of free (unbound) TCDD in arterial blood (mol/m^3)

CV_{LIVER} = concentration of TCDD in venous blood exiting liver (mol/m^3)

AM = amount of TCDD excreted or metabolized by microsomes in liver (mol)

AP = amount of TCDD in peritoneal cavity available for absorption (mol)

KAB = TCDD equilibrium blood binding constant (unitless)

KA = absorption constant from gastrointestinal tract into liver (1/hour)

KFC = first order metabolic rate constant (1/hour)

Dose = Administered dose (mol)

BW = body weight (kg)

V_{LIVER} = volume of liver (m^3)

R_{LIVER} = liver/blood partition coefficient (unitless)

$BM1$ = TCDD binding capacity to cytosolic protein (mol)

$KB1$ = TCDD binding constant to cytosolic protein (mol/m^3)

$BM2$ = TCDD binding capacity to microsomal protein (mol)

$KB2$ = TCDD binding constant to microsomal protein (mol/m^3)

$BM2_{non}$ = binding capacity to microsomal protein, noninduced only (mol)

$BM2_{ind}$ = binding capacity to microsomal protein, induced only (mol)

Blood Compartment

$$dA_{BLOOD}/dt = (Q_{FAT} * CV_{FAT}) + (Q_{LIVER} * CV_{LIVER}) + (Q_{SLOW} * CV_{SLOW}) + (Q_{RICH} * CV_{RICH}) - (Q_{BLOOD} * CA) + (dAP/dt) * (KAB / (1 + KAB))$$

$$C_{BLOOD} = A_{BLOOD} / V_{BLOOD}$$

$$CA = C_{BLOOD} / (1 + KAB)$$

A_{BLOOD} = total amount of TCDD in blood (mol)

Q_{BLOOD} = total cardiac output (m^3 /hour)

C_{BLOOD} = concentration of free and bound TCDD in blood (mol/m^3)

V_{BLOOD} = volume of blood compartment (m^3)

Q_{FAT} = blood perfusion (flow) through fat compartment (m^3 /hour)

Q_{SLOW} = blood perfusion (flow) through slowly perfused compartment (m^3 /hour)

Q_{RICH} = blood perfusion (flow) through richly perfused compartment (m^3 /hour)

CV_{FAT} = concentration of TCDD in venous blood exiting fat compartment (mol/m^3)

CV_{SLOW} = concentration of TCDD in venous blood of slowly perfused tissue (mol/m^3)

CV_{RICH} = concentration of TCDD in venous blood of richly perfused tissue (mol/m^3)

Fat, Richly Perfused and Slowly Perfused Compartments

$$dA_i/dt = Q_i * (CA - CV_i)$$

$$CV_i = A_i / (V_i * R_i)$$

i = fat, richly perfused, and slowly perfused (FAT, RICH, SLOW) compartments

CV_i = venous blood concentration leaving compartment i (mol/m^3)

Q_i = blood perfusion rate through compartment i (m^3 /hour)

R_i = partition coefficient (ratio) between compartment i and blood (unitless)