NATIONAL INSTITUTE OF PUBLIC HEALTH AND THE ENVIRONMENT
BILTHOVEN, THE NETHERLANDS

Report no. 604138.001

Modeling of Ah-receptor dependent P450 induction I. Cellular model definition and its incorporation in a PBPK model of 2,3,7,8-TCDD

M.J. Zeilmaker and J.C.H. van Eijkeren

November 1997

This study has been performed on the authority and for the account of the Directorate-General of RIVM within the framework of project number 604138, Extrapolation to Man.

National Institute of Public Health and the Environment, P.O. Box 1, 3720 BA Bilthoven, The Netherlands,
telephone +31(0)30 274911, telefax +31(0)30 2742971

Mailing list

1-5 Directie RIVM
6 Dr. M.E. Andersen, ICF Kaiser Engineeers, Research Triangle Park, USA
7 Dr. M. Van den Berg, RITOX, Rijksuniversiteit Utrecht
8 Dr. L. Birnbaum, US EPA, Research Triangle Park, USA
9 Dr. J. Borm, Milieu en Gezondheidswetenschappen, Rijksuniversiteit Limburg
10 Dr. B. Brouwer, Vakgroep Toxicologie, Landbouwuniversiteit Wageningen
11 Dr. G. Carrier, Faculté de médecine, Université de Montréal, Canada
12 Dr. R. Conolly, Central Institute of Industrial Toxicology, Research Triangle Park, USA
13 Dr. P. Doucet, Vakgroep Theoretische Biologie, VU Amsterdam
14 Dr. Ir. R. Hoogenboom, RIKILT, Wageningen
15 Dr. J. de Jongh, RITOX, Rijksuniversiteit Utrecht
16 Dr. D. Paustenbach, McLaren-Hart, Almeda, USA
17 Prof. Dr. W. Seinen, RITOX, Rijksuniversiteit Utrecht
18 Dr. D. Schrenk, Institute of Toxicology, University of Tübingen, Germany
19 Dr. J.A. van Zorge, DGM/SVS, Afd. Normstelling
20 Depot Nederlandse Publicaties en Nederlandse Bibliografie
21 Dr. Ir. H.J.G.M. Derks, Hoofd LGO
22 Dr.Ir. E. Lebret, Hoofd LBO
23 Prof. Dr. C.J. van Leeuwen, CSR
24 Dr. A.K.D. Liem, LOC
25 Dr. Ir. G. de Mik, Directeur Sector 4
26 Dr. W. Slob, LEO
27 Dr. M.P. van Veen, LBO
28 Prof. Dr. J.G. Vos, Hoofd LPI
29 SBD/Voorlichting en Public Relations
30 Bibliotheek RIVM
31 Bureau Rapportenregistratie
32-33 Auteurs
34 Archief LBO
35-55 Bureau Rapportenbeheer

Contents

Summary

The interspecies extrapolation of chemical toxicity is traditionally based on the daily administered dose of the chemical. However, in the case of compounds with strong bioaccumulating properties, such as 2,3,7,8-TetraChloroDibenzo-p-Dioxin (TCDD), chronic toxicity is expected to scale better with the amount of TCDD in the body than with the daily administered dose.

In order to use the amount of TCDD in the body as the starting point for the extrapolation of TCDD toxicity quantification is needed of the accumulation of TCDD in the body in animals and man. When only the lipophilic properties of TCDD are taken into consideration a linear relationship is expected between the fat content of the body, the daily administered amount of TCDD and the amount of TCDD in the body. However, in animals the disposition mechanism of TCDD is more complex. For example, in the rat the exposure to TCDD leads, as dose is increased, to accumulation in the liver over the adipose tissue. The explanation of this observation is as follows. In accordance with its lipophilic nature TCDD accumulates, at relative low doses, in hepatic fat. However, the rat liver possesses a second, dose dependent, uptake mechanism for TCDD. For, in the liver cell TCDD may bind to the Ah-receptor, a receptor protein regulating the expression of P450 genes. As a result the amount of P450 proteins in the cell increases. In turn this may lead to an increased binding of TCDD to the induced proteins. In the case of compounds with a high P450 induction potency but relative slow metabolism, as is the case with dioxins, this mechanism leads to the accumulation of the dioxin-P450 complex in the cell.

In order to incorporate the above described disposition mechanism into the safety evaluation of TCDD we developed a generic model for the interactions of ligands (dioxins, polyaromatic hydrocarbons) with the Ah-receptor in the cell. This cellular model contains the binding of the ligand to the Ah-receptor, the binding of the ligand-Ah-receptor complex to specific DNA binding domains (Xenobiotic Responsive Elements (XRE)), Ah-receptor dependent *de novo* P450 synthesis and the binding of the ligand to induced P450 proteins together with their metabolism. The Ah-receptor/P450 induction model was incorporated in a Physiologically Based PharmacoKinetic (PBPK) model of the rat. This rat PBPK model was calibrated and validated for TCDD.

The PBPK model presented here will be scaled from the rat to man. The human PBPK model will be used to calculate a safe human exposure levels to TCDD. These exposure level, which is presented in a separate report, will be compared with the Tolerable Daily Intake of TCDD as calculated with similar modeling techniques by WHO and, more recently, the Health Council of the Netherlands.

Samenvatting

De extrapolatie van de toxiciteit van chemische stoffen van proefdieren naar de mens vindt traditioneel plaats op basis van de dagelijks toegediende hoeveelheid van een stof. Echter, voor stoffen met sterk accumulerende eigenschappen zoals 2,3,7,8-TetraChloroDibenzo-p-Dioxine (TCDD) ligt een extrapolatie op basis van de hoeveelheid van de stof die zich in het lichaam opgehoopt heeft meer voor de hand.

Het uitvoeren van een dergelijke extrapolatie vereist het kwantificeren van de ophoping van TCDD in het lichaam. Wanneer ervan uitgegaan wordt dat TCDD zich uitsluitend in lichaamsvet ophoopt wordt een lineare relatie verwacht tussen de hoeveelheid lichaamsvet, de dagelijkse blootstelling aan TCDD en de hoeveelheid TCDD in het lichaam. In proefdieren blijkt de ophoping van TCDD echter niet (alleen) volgens dit mechanisme te verlopen. In de rat b.v. concentreert TCDD zich bij relatief hoge blootstelling in de lever, en niet in het vetweefsel. De verklaring voor deze bevinding is als volgt. Bij relatief lage blootstelling hoopt TCDD zich, in overeenstemming met zijn lipofiele karakter, op in het vet van de lever. De rattenlever bezit echter nog een tweede opnamemechanisme voor TCDD. In de cel kan TCDD zich aan de Ah-receptor binden. Deze binding leidt tot een verhoogde expressie van P450 genen. Hierdoor neemt de concentratie van P450 eiwitten in de cel sterk toe. Daar TCDD voor een aantal van deze eiwitten een hoge bindingsaffiniteit bezit leidt de toename van P450 eiwitten in de cel tot een verhoogde vorming van TCDD-P450 complexen. In het geval van stoffen met een hoog P450 inducerend vermogen maar relatief langzaam metabolisme, zoals met dioxinen het geval is, leidt dit mechanisme tot de ophoping van het dioxine-P450 complex in de cel.

Om het boven beschreven accumulatiemechanisme in de risicoschatting van TCDD op te nemen is een algemeen geldend model voor de interacties van liganden (dioxinen, maar ook polycyclische aromatische koolwaterstoffen) met de Ah-receptor in de cel ontwikkeld. Het model bevat de binding van de ligand aan de Ah-receptor, de binding van het ligand-Ah-receptor complex aan specifieke DNA bindingssequenties ("Xenobiotic Responsive Elements"), Ah-receptor afhankelijke *de novo* P450 synthese en de binding van de ligand aan de geinduceeerde P450 eiwitten, inclusief het metabolisme van de ligand. Dit Ah-receptor P450 inductie model werd opgenomen in een fysiologisch georiënteerd farmacokinetisch model ("PBPK model") voor de rat. Dit ratten PBPK model is voor TCDD gekalibreerd en gevalideerd.

Het in dit rapport beschreven ratten PBPK model zal naar de mens geschaald worden. Vervolgens zal het humane PBPK model gebruikt worden om het voor de mens veilig geachte blootstellingsniveau van TCDD te berekenen. Dit blootstellingsniveau zal vergeleken worden met de, middles vergelijkbare methoden, door de WHO en, meer recent, de Gezondheidsraad berekende "Tolerable Daily Intake" voor TCDD.

1. Introduction

In mammals the exposure to dioxins and polycyclic aromatic hydrocarbons (PAHs) leads to interactions with the Ah-receptor, a protein which regulates gene expression in the cell. One group of genes which is regulated by the Ah-receptor is the group of P450 genes (Landers and Bunce, 1991). P450 genes code for proteins which metabolise a wide variety of endogenous and foreign compounds. As a result of the P450 induction process an efficient metabolism, and subsequent removal from the cell, of P450 inducers is expected. However, in the case of compounds with high P450 induction potency but relatively slow P450 metabolism, such as dioxins, this mechanism leads to the accumulation of the P450 inducing agent in the cell. In the case of PAHs the P450 induction mechanism does lead to the efficient removal of these compounds from the cell. However, as PAH metabolites often display a vigorous reactivity towards proteins and DNA this occurs at the expense of a, sometimes irreversible, toxic response (point mutation).

Until now no methods are available which make the incorporation of the interactions of dioxins and PAHs with the Ah-receptor into the risk assessment of these compounds possible. PBPK modeling however offers a good opportunity to achieve this goal. In general PBPK models describe the uptake, the distribution, the metabolism and the removal of chemicals from the body within a physiological framework of the organism, i.e. organs which exchange chemicals and their metabolites via the blood. In this report we present a cellular model for the interactions of dioxins and PAHs with the Ah-receptor and with Ah-receptor dependent P450 proteins. This model contains the binding to the Ah-receptor, the binding of the dioxin/PAH-Ah-receptor complex to specific DNA domains (Xenobiotic Responsive Elements (XRE)), Ah-receptor dependent *de novo* P450 synthesis and the binding of dioxins/PAHs to induced P450 proteins together with their eventual metabolism. The cellular model was implemented in a rat PBPK model. In the case of the dioxin 2,3,7,8-TetraChloroDibenzo-*p*-Dioxin (TCDD) this rat model was calibrated and validated using *in vivo* data on the accumulation of TCDD in the cell, especially the liver cell, and its P450 inducing properties.

2. Model definition and model parameters

2.1 Cellular model for Ah-receptor dependent P450 induction

The model concept for Ah-receptor dependent P450 induction is presented in Fig. 1. The P450 enzyme machinery is assumed to have two components: a non-Ah-receptor dependent, basal concentration of P450 enzymes and an Ah-receptor regulated concentration of P450 enzymes. After entering the cell the Ah-receptor ligand L may either be taken up in cellular fat or bind to the Ah-receptor or basal and induced P450 enzymes. The ligand-Ah-receptor complex binds to Xenobiotic Responsive Elements. The formation of the ligand-Ah-receptor-XRE complex leads to induced P450 transcription, i.e. formation of P450 mRNA, and, hence, increased P450 protein levels in the cell.

In order to avoid parameter estimation problems in modeling this mechanism several assumptions had to be made. First we assumed that the ligand-Ah-receptor complex binds with equal efficiency to XRE sequences which are located at various Ah-receptor regulated P450 genes. Second, we assumed that the efficiency with which a particular ligand-Ah-receptor-XRE complex enhances P450 transcription cq. P450 translation is equal for all Ah-receptor regulated P450 genes. Furthermore, we assumed that the uptake of the Ah-receptor ligand in the cellular fat fraction, the binding of the ligand to the Ah-receptor and to basal and induced P450 proteins, the binding of the ligand-Ah-receptor complex to XRE sequences and induced mRNA synthesis reaches equilibrium instantaneously upon variations in the total cellular ligand concentration. For induced P450 transcription this however was not the case.

The model for Ah-receptor dependent P450 induction in the cell and its implementation in the liver compartment of a PBPK model is given in the Appendix.

2.2 Rat PBPK model of TCDD

For the description of the fate of TCDD in rodents a five compartment PBPK model was chosen. The compartments of this model are the central blood compartment, and the four peripheral compartments adipose tissue, slowly and richly perfused tissue and liver.

The distribution of TCDD in all compartments but adipose tissue, was modeled as a plasma compartment partitioning based on the plasma's and the organ's fat and water fractions (see Appendix). In this it was assumed that the fraction of exchangeable TCDD in blood and tissue interstitial space is 1. In addition the distribution of TCDD in the liver compartment was modeled to occur also by binding to the Ah-receptor and to basal and Ah-receptor dependent induced P450 proteins. In the rodent liver the proteins P450 1A1 (1A1)

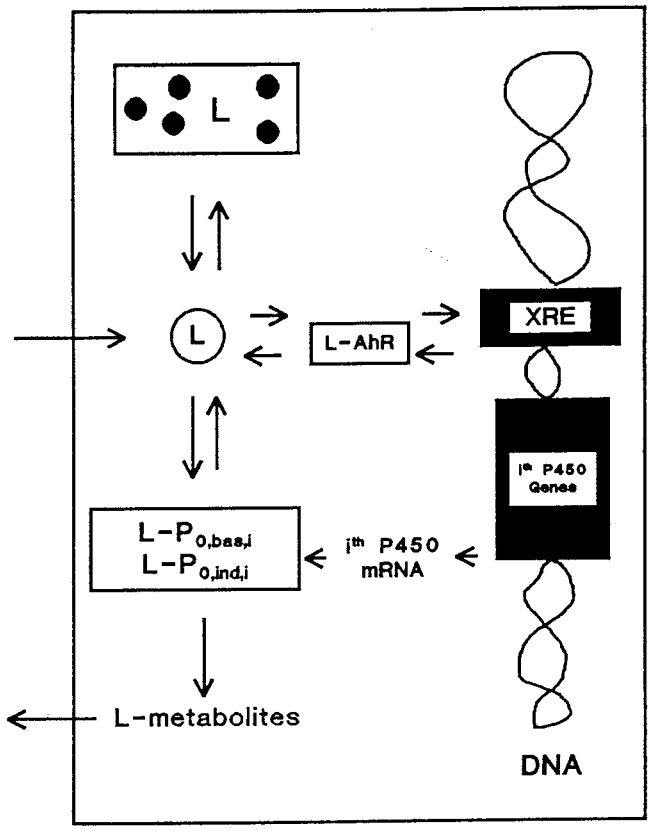


FIG. 1. Model for Ah-receptor dependent P450 induction in the cell. L: Ah-receptor ligand; L-AhR: ligand-Ah-Receptor complex; XRE: Xenobiotic Responsive Element; $L\text{-}P_{0,bas,i}$ and $L\text{-}P_{0,ind,i}$: Ligand -P450 protein complexes. ●: cellular fat.

and P450 1A2 (1A2) are induced with almost equal efficiency by TCDD (Tritscher *et al.*, 1992). However, only the induction of the 1A2 protein was found to correlate with the accumulation of TCDD in the rodent liver (Poland *et al.*, 1989). The 1A2 protein was therefore considered as the most important P450 binding site for TCDD in the liver.

The distribution in the liver and the slowly and the richly perfused compartments was modeled as perfusion limited. In previous studies it was found that the uptake of TCDD by the fat compartment should be modeled as a diffusion limited instead of a perfusion limited process (Derks *et al.*, 1993). This was achieved by subdividing this compartment into a "blood" and a "tissue" compartment and modeling mass transport between these two governed by Fick's law. In rodents the elimination of TCDD from the body predominantly occurs by P450 metabolism (Gasiewicz *et al.*, 1983; Kedderis *et al.*, 1991). This elimination is relatively slow. For example reported terminal half-lives of TCDD in the rat varied between 12 and 31 days (Van den Berg *et al.*, 1994). This indicates that the elimination of TCDD does not depend on the distribution kinetics of this compound. Under such conditions

the terminal elimination is expected to scale linearly with the intrinsic hepatic clearance of TCDD. As such the latter entity depends linearly on the amount of the TCDD metabolising protein. In this context the hepatic enzyme 1A1 is a likely candidate for P450 mediated elimination of TCDD in the rat and in man (Tai *et al.*, 1993). However, in rodents the elimination of TCDD from the body does not scale with the amount of 1A1 in the liver. For example, in rodents hepatic 1A1 may be induced many-fold by TCDD. However, in animals having substantially increased hepatic levels of 1A1 the elimination of TCDD was reported to be induced only two-fold (Leung *et al.*, 1988) or even less then two-fold (Kedderis *et al,* 1991). This finding practically excludes 1A1 as the enzyme which is responsible for the elimination of TCDD in rodents. As the hepatic enzyme 1A2 is induced as efficiently in the rodent liver as the 1A1 enzyme (see for example Tritscher *et al.*, 1992) a similar conclusion can be drawn for 1A2. In the model the metabolism of TCDD was therefore not modeled as a 1A1 or 1A2 mediated mechanism but as a mechanism which depends on P450 activity other than 1A1 and 1A2. In concordance with the observed inducibility of TCDD elimination in rodents this activity was assumed to be Ah-receptor regulated.

Both 1A1 and 1A2 were assumed to contribute to the cellular Ethoxyresorufin-O-dealkylase activity (EROD). EROD activity was modeled as a linear function of the cellular concentrations of these enzymes (see Appendix).

2.3 Model parameters of the rat PBPK model

The physiological constants were obtained from the Physiological Parameter Values for PBPK models as prepared by the International Life Sciences Institute (ILSI)/ Risk Science Institute (RSI) (1994). Animal growth was modeled as follows. In the study of Abraham *et al.* (1988) no specific information was given on the growth of the animals (only the body weight at the start of the experiment was given). In this study the growth of the animals was therefore modeled in accordance with the average growth of the rat as reported by Van Zutphen *et al.* (1991). Animal growth in the studies of Kociba *et al.* (1978) and Tritscher *et al.* (1992) were modeled in accordance with Lucier *et al.* (1991) and Kociba *et al.* (1976). Normal growth was corrected for the increase of the relative gain of weight of the liver which occurs as a result of liver hypertrophy in animals which are exposed to TCDD (Tritscher: relative liver weight, controls 0.03, 100 ng TCDD/kg/day: 0.042; Kociba: relative liver weight, controls 0.025, 100 ng TCDD/kg/day: 0.033). It was assumed that liver hypertrophy increases linearly with the exposure time to TCDD. The absorption coefficient for the oral route was obtained from Leung *et al.* (1990) and the absorption coefficient for the subcutanous route from Abraham *et al.* (1988). The coefficients for the partitioning of TCDD between liver, slowly and richly perfused and the adipose compartment respectively and the plasma were obtained as follows. At relative low fat content of a particular organ the water/fat partition coefficient of that organ

relative to plasma scales approximately linear to that fat content (see Appendix). The partition coefficient of the liver and the slowly and the richly perfused organ compartments were therefore scaled in proportion to their fat content, The fat content of these organs was obtained from (Van der Molen *et al.*, 1996). For the adipose tissue the aformentioned approximation does not hold. Given a fat content of 80-100% the partition coefficient of the adipose tissue is expected to exceed 500. The actual value of this coefficient was determined by fitting the model to experimental data (see below). The concentration of the Ah-receptor in the liver was obtained from Kohn *et al.* (1994). The binding constant of TCDD to P450 enzymes other than 1A2 was set equal to TCDD's binding constant to induced hepatic P450 microsomal binding sites (Poland *et al.*, 1989). The maximal inducible synthesis rate for 1A1 and 1A2 in the rodent liver were calculated from the data of Tritscher *et al.* (1992).

The partition coefficient of the adipose tissue, the parameter for the efficiency of the P450 induction process, the effective dissociation constant for TCDD to hepatic 1A2, the diffusion constant for the transport of TCDD from blood to adipose tissue and the factor relating the 1A1 and 1A2 concentrations to hepatic EROD activity were obtained by fitting the model to experimental data.

A complete list of model parameter values is given in the Appendix.

3. Model simulations

For the estimation of parameters by fitting of the model to experimental data the study of Abraham *et al.* (1988) was selected. The study of Abraham consisted of two parts, a time-course study and a dose-response study. In the time-course study the disposition of TCDD in liver and adipose tissue and the EROD induction in the liver were measured at various time points after the administration of a single subcutanous dose of 300 ng/kg TCDD. In the dose-response experiment the disposition of TCDD was determined in the liver and the adipose tissue of rats at 168 hr after the administration of a single subcutanous dose. In this study the administered dose varied from 1 to 3000 ng/kg.

3.1 Acute exposure

In rodents the P450 proteins which are responsible for TCDD metabolism are not known. Though the hepatic P450 proteins 1A1 and 1A2 are likely candidates for the elimination of TCDD from rodents the inducibility of TCDD metabolism does not scale in accordance with the induction of these proteins. In concordance with this finding we obtained no adequate simulations of the time-course data of the Abraham study with a PBPK model in which TCDD metabolism depended on the hepatic concentration of 1A1, 1A2 or a combination of these two proteins (data not shown). Further studies revealed that these data

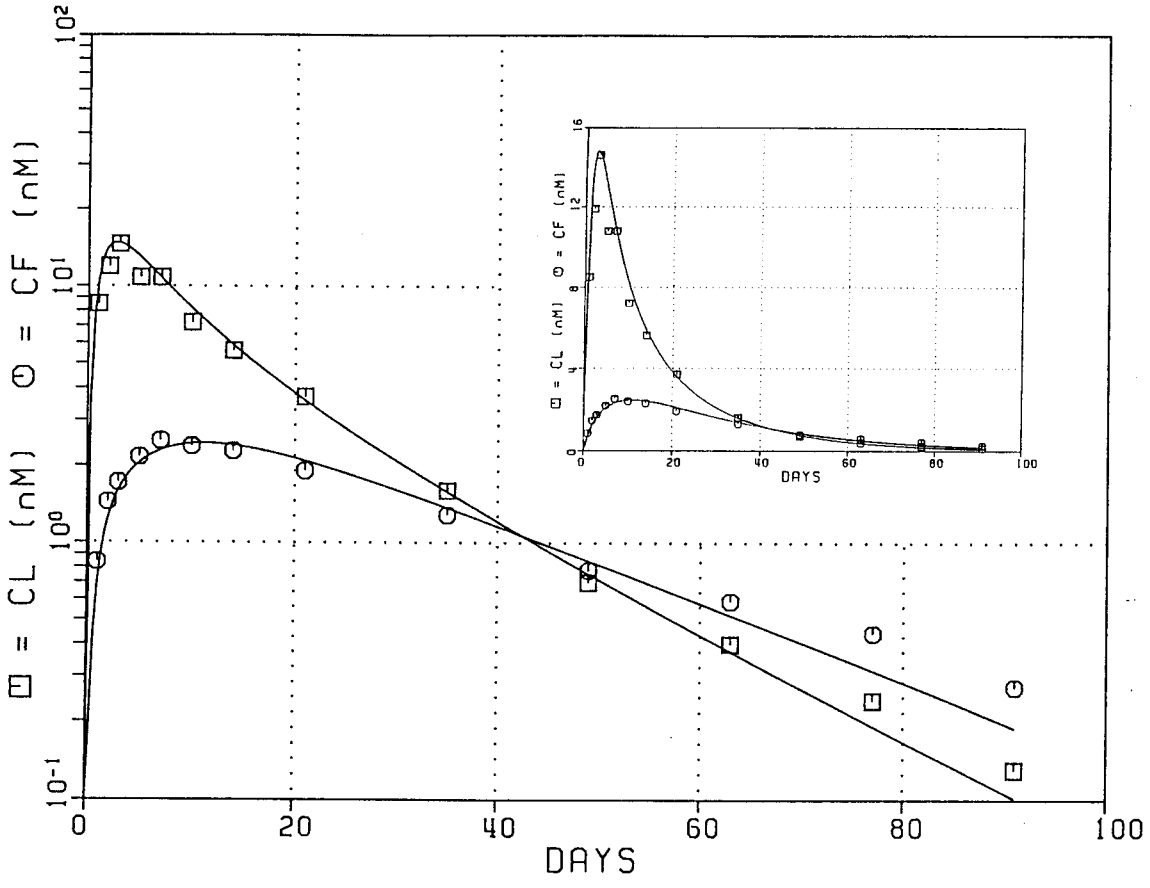
were only compatible with first-order, low inducible TCDD metabolism with a terminal half-life of 18 days (2-4 fold induciblity, to be compared with an inducibility of 242 for 1A1 and 8 for 1A2). This finding is in agreement with the reported terminal half-life of TCDD of 16-21 days and a two-fold inducibility of TCDD metabolism in rodents.

In the model TCDD mediated Ah-receptor dependent P450 induction proceeds via a two-step mechanism. First TCDD binds to the Ah-receptor. The formed complex then binds to XRE. In the model the efficiency by which TCDD induces, via the binding to the Ah-receptor and the binding of the TCDD-Ah-receptor complex to XRE, *de novo* P450 synthesis is expressed by only one parameter, i.e. μ. Given the time-course data of the Abraham study μ appeared to be well identifiable, i.e. one value of μ could be found which led to an optimal description of the uptake of TCDD in the liver.

Fig. 2a shows the disposition of TCDD in the liver and the adipose tissue in the time-course part of the Abraham study as simulated by the calibrated model. In accordance with the experimental observations the model simulated a preferential accumulation of TCDD in the liver over the adipose tissue. Simulated TCDD concentrations in the liver quickly rose until a maximum which was reached 7 days after administration. Thereafter the concentration of TCDD in the liver gradually declined and eventually became lower than its concentration in the adipose tissue. The calibrated model was (partially) validated on the induced hepatic EROD activity. As shown in Fig. 2b the simulated EROD activity in the liver showed an almost similar time dependent pattern as the concentration of TCDD in the liver. This result indicates that the model was well able to simulate 1A1 and 1A2 induction in the liver.

The model was further validated on the results of the dose-response part of the Abraham study. Fig. 3 shows the result of this validation study, i.e. without changing its parameter values, the experimental observations were simulated. The calibrated model accurately simulated the concentrations of TCDD in the liver and the adipose tissue. On the mean the ratio between the the simulated and the measured concentrations in the liver and the adipose tissue were 1.2 (range: 1.0-1.4) and 1.1 (range: 0.9-1.7).

2A



2B

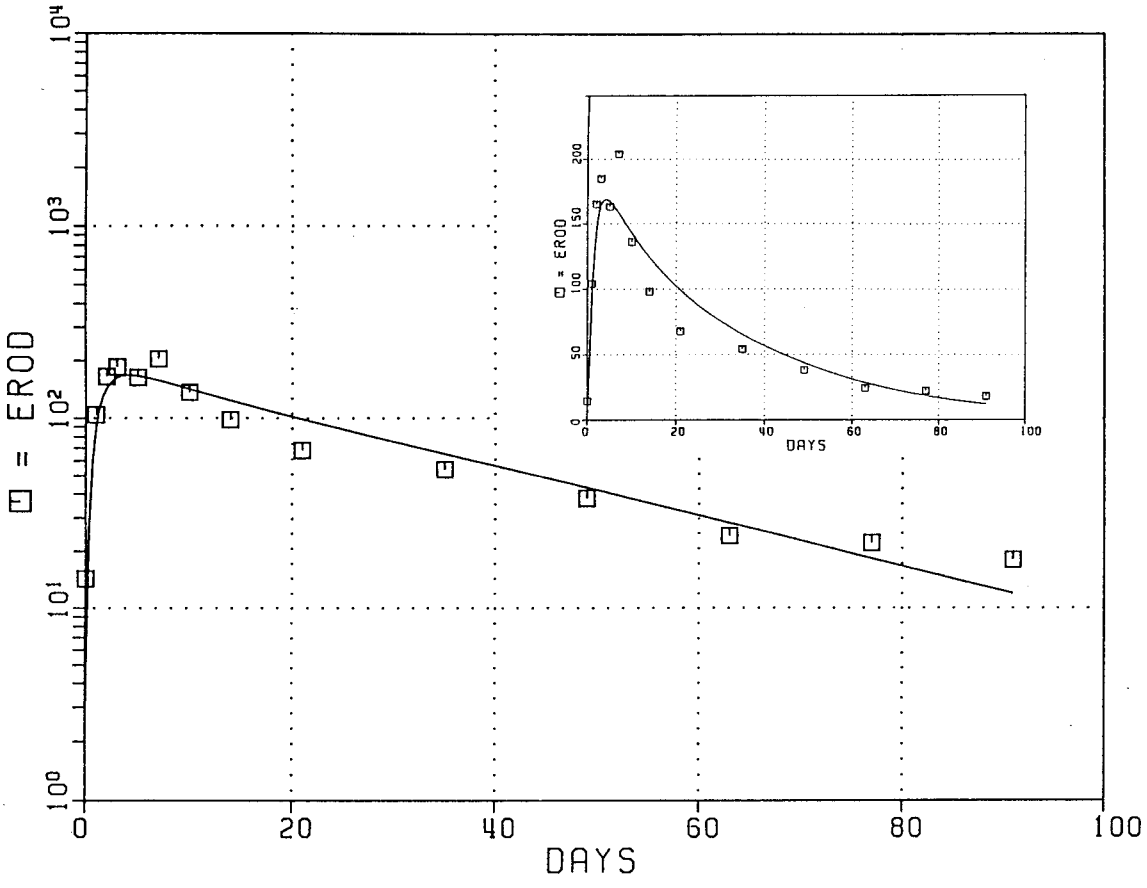
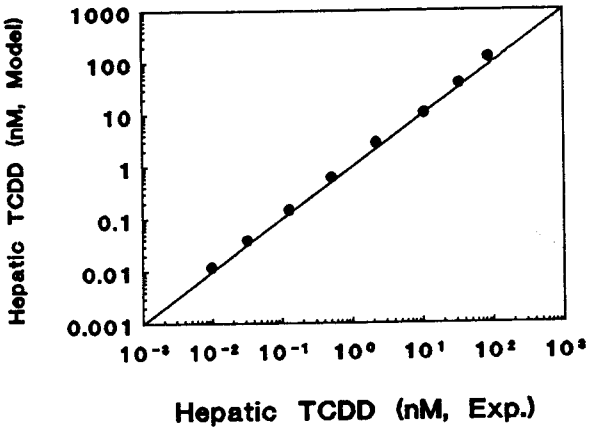


FIG. 2. The model calibrated to the time-course data of Abraham *et al.*, (1988). A: Ordinate: TCDD concentrations in whole liver (C_l) and adipose tissue (C_f). B: Ordinate: EROD activity in the liver. Abscissa: Time after administration of TCDD. Insert: linear scale.

In calibrating the model the criterium of weighted least squares was used to fit the model to experimental observations. As weights standard deviations of the concentrations of TCDD in the liver and the adipose tissue respectively were used. Estimated model parameters: partition coefficient of the adipose tissue, the dissociation constant for the binding of TCDD to 1A2, the diffusion constant for the transport of TCDD from blood to adipose tissue, the dissociation constant for the binding of TCDD to the Ah-receptor and the dissociation constant for the binding of the TCDD-Ah-receptor complex to XRE. (B) Ordinate: EROD activity in whole liver tissue. Insert: Linear scale. Estimated model parameters: the factor relating the 1A1 and 1A2 concentrations to hepatic EROD activity.

3A



3B

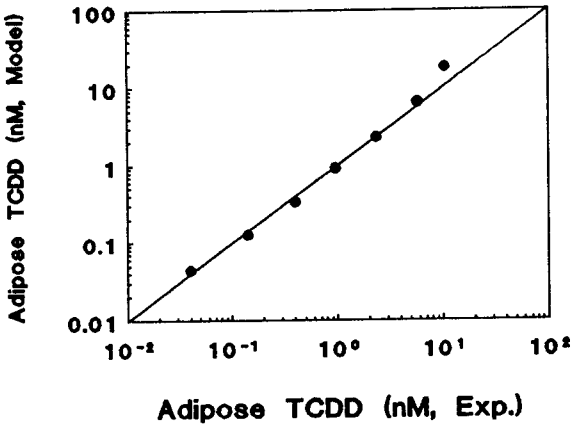


FIG. 3. Model simulation of the concentration of TCDD in the liver and the adipose tissue as observed in the study of Abraham *et al.* (1988) in rats 168 hr after the subcutanous administration of a single dose of 1-3000 ng/kg TCDD. Ordinate: Simulated TCDD concentration. Abscissa: Experimentally measured TCDD dose (A) Liver (B) Adipose tissue. The line shows identity of simulated and measured TCDD concentrations. Model parameters were as described in Fig. 2.

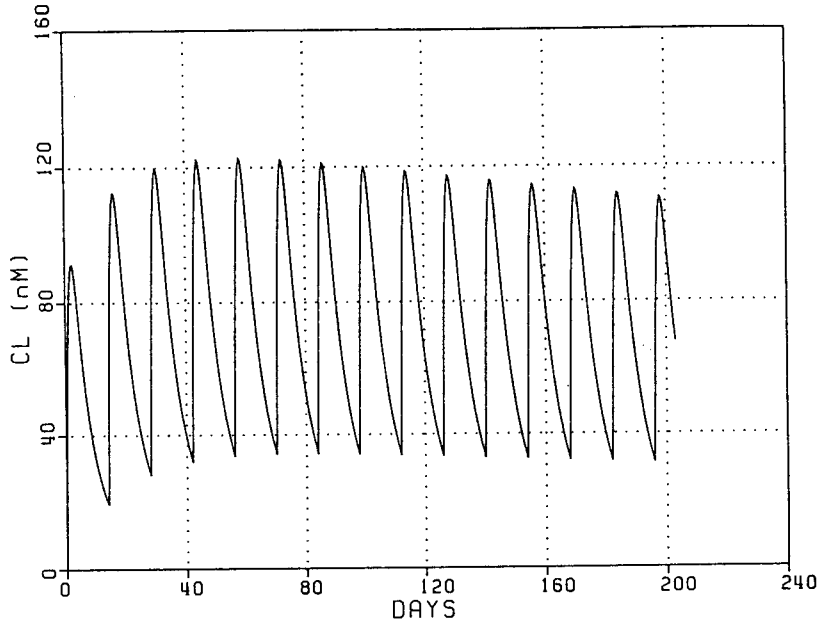
3.2 Chronic exposure

The model's ability to simulate the disposition of TCDD in rodents was also investigated after chronic exposure. For this purpose two studies were selected. In the first study rats received biweekly oral TCDD doses of 50, 150, 500 and 1750 ng/kg for a period of 30 weeks (Tritscher *et al.,* 1992). One week after the administration of the last dose the concentration of TCDD and the concentrations of the proteins 1A1 and 1A2 were measured in the liver. In the second study, performed by Kociba *et al.* (1978), rats were dosed for two years to TCDD doses of 1, 10 and 100 ng/kg/day. At the end of this period the concentrations of TCDD in the liver and the adipose tissue were measured.

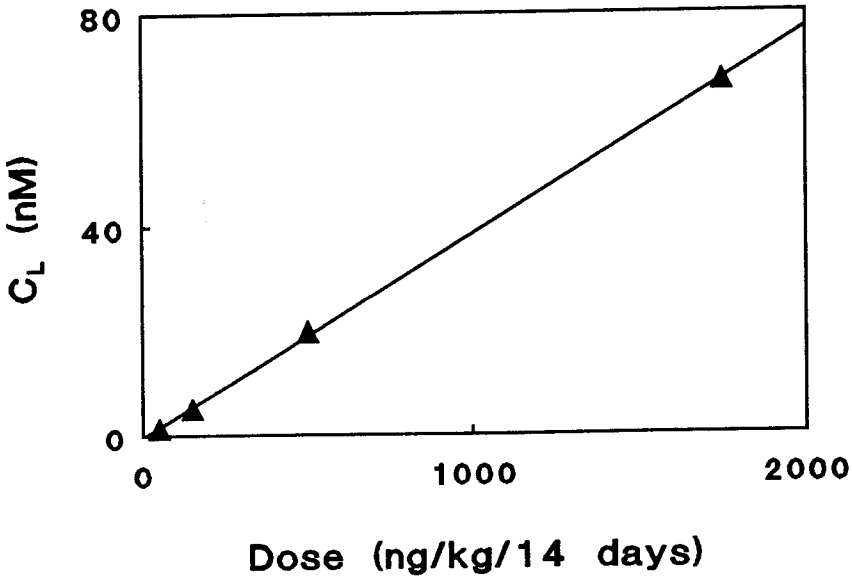
In simulating the data of the Tritscher study it was found that the model which was calibrated on the time-course part of the Abraham study, i.e. acute TCDD exposure, systematically overestimated the hepatic concentration of TCDD. Sensitivity analysis identified a too low metabolism of TCDD as the most likely cause for this model behaviour. A two-fold increase of TCDD metabolism led to a model which described the disposition in the liver quite well. The result of this recalibration is shown in Fig. 4. Figure 4 also shows that the dose-schedule as applied in the Tritscher study leads to TCDD concentrations in the liver and the adipose tissue which, though variable, center around a plateau value. In the liver this plateau slowly, but steadily, decreases as exposure advances. This model behaviour is caused by the increased capacity of the liver to metabolize TCDD, a feature resulting from the modeled liver hypertrophy. The (re)calibrated model was validated on the 1A1 and 1A2 induction data in the liver at the end of the exposure period. The result of this validation study, which is shown in Fig. 5, indicates the high accuracy with which the model predicts 1A1 and 1A2 induction after chronic exposure to TCDD.

Fig. 6 shows the simulation of the results of the Kociba study by the model which was calibrated on the time-course part of the Abraham study. The model underestimated, especially at the two lowest dose levels tested, the concentrations of TCDD in the liver as well as in the adipose tissue. In contrast to the Tritscher study the recalibration of the model did not lead to an adequate description of the entire data set. For example, at the lowest dose level tested, i.e. 1ng/kg/day, the affinity of the liver and the adipose tissue for TCDD had to be increased by a factor of two resp. four in order to describe the disposition of TCDD in these organs well. However, at the highest dose level tested this recalibration led to a two resp. four fold overestimation of the amounts of TCDD in the liver and the adipose tissue.

4A


160
120
80
40
0
CL (nM)
0
40
80
120
160
200
240
DAYS

4B


80
40
0
C_L (nM)
0
1000
2000
Dose (ng/kg/14 days)

4C

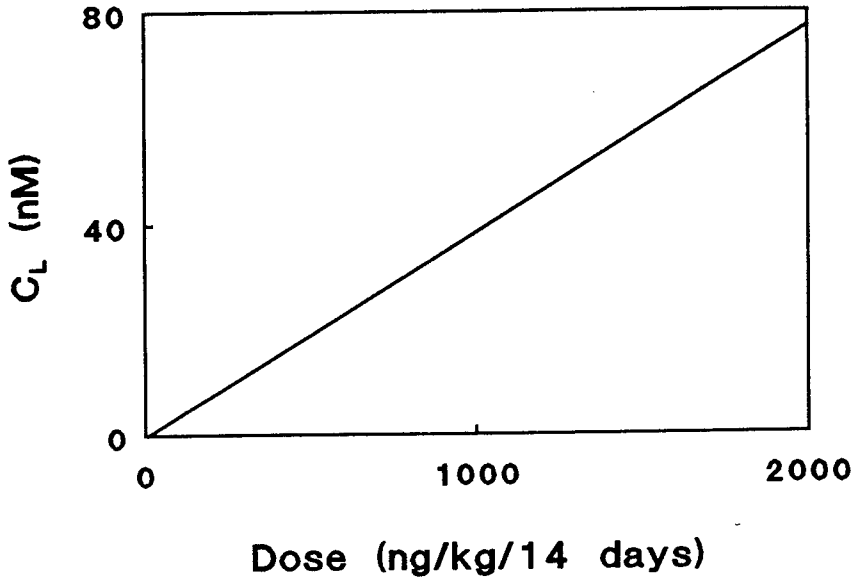


FIG.4. The model calibrated to the study of Tritscher *et al.* (1992). A: Simulation of the time-course of the concentration of TCDD in the liver (C_l) and (C_f) in rats which have been treated for 30 weeks to biweekly doses of 1750 ng/kg. Ordinate: Simulated TCDD concentration. Abscissa: Time in days B and C: the measured (B) and simulated (C) concentration of TCDD in the liver (C_l) in rats which have been treated for 30 weeks with biweekly doses of 50, 150, 500 and 1750 ng/kg. Ordinate: Simulated TCDD concentration. Abscissa: Administered TCDD dose rate.

The model parameters were as described in Fig. 2, except for the inducibility of TCDD metabolism (Fig. 2: two-fold inducibility of TCDD metabolism, this figure: four-fold inducibility of TCDD metabolism). Data: Tritscher *et al.*(1992).

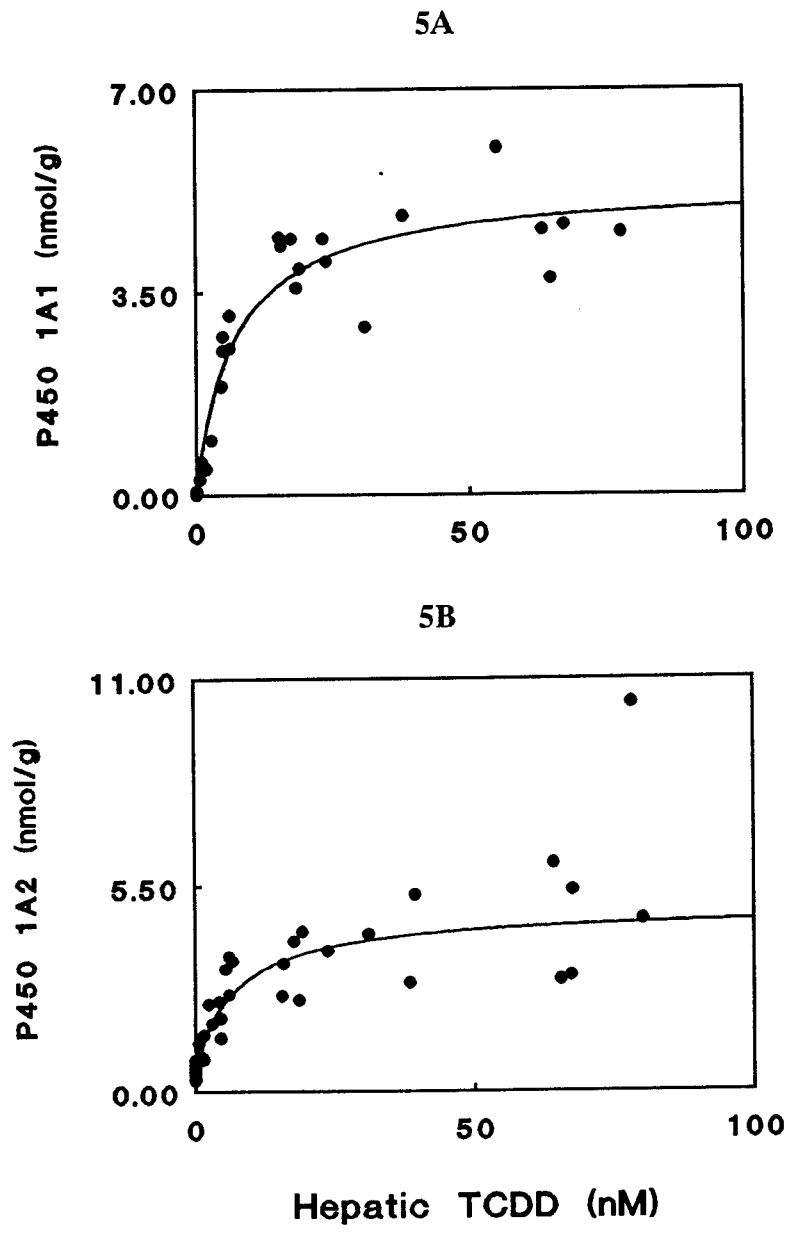
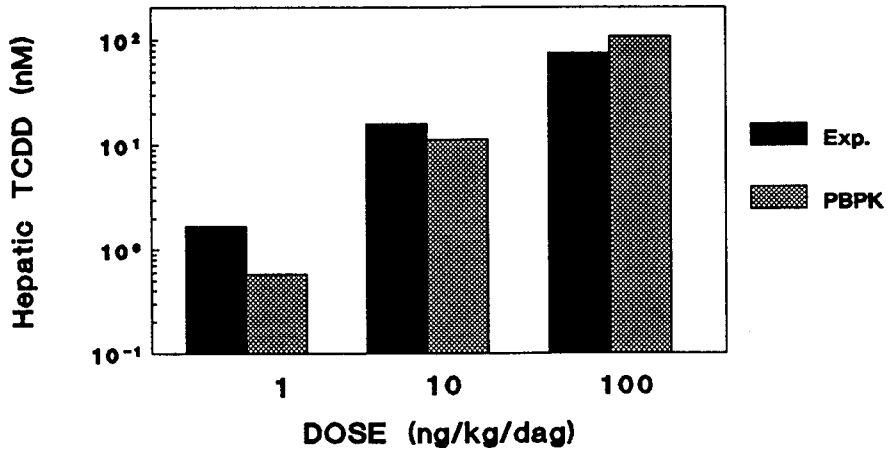


FIG. 5. Model simulation of the dose-response relationship of the concentration of TCDD vs. the concentration of 1A1 (A) and 1A2 (B) in the liver of rats exposed for 30 weeks to biweekly TCDD doses of 50, 150, 500 and 1750 ng/kg. Ordinate: 1A1 and 1A2 concentrations (nmol/g). Abscissa: Hepatic TCDD concentration (nM).

The model parameters were as described in Fig. 2, except for the inducibility of TCDD metabolism (Fig. 2: two-fold inducibility of TCDD metabolism, this figure: four-fold inducibility of TCDD metabolism). Data: Tritscher *et al.*, (1992).

6A



6B

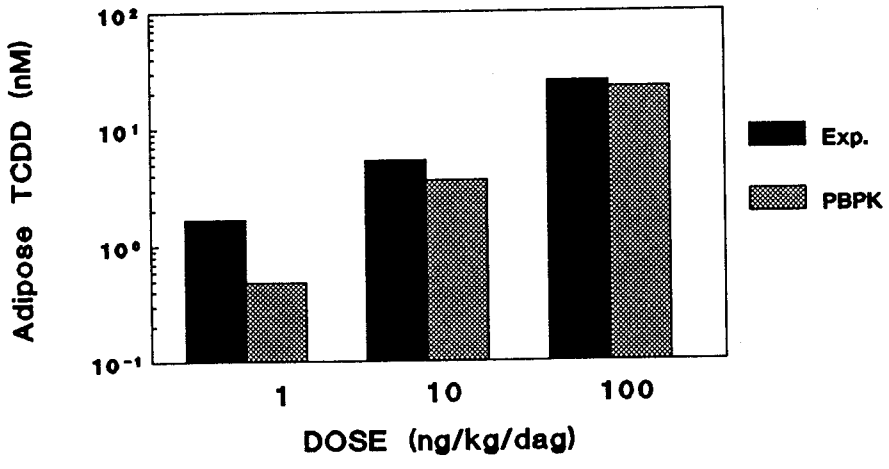


FIG. 6. Model simulation of the concentration of TCDD in the liver (A) and the adipose tissue (B) of rats exposed via the food for two years to TCDD doses of 1, 10 and 100 ng/kg/day.

The model parameters were as described in Fig. 2 except for the fraction of TCDD which was taken up with the food (F_{abs} = 1). Data: Kociba *et al.*, (1978).

4. Discussion

The interspecies extrapolation of chemical toxicity is traditionally based on the administered amount of the chemical. However, in the case of compounds with strong bioaccumulating properties, such as TCDD, chronic toxicity is expected to scale better with the amount of TCDD accumulated in the body than with the daily administered dose. It has therefore been proposed to base the extrapolation of chronic TCDD toxicity on the amount accumulated in the body (Ahlborg *et al.*, 1992; Andersen *et al.*, 1993, Aylward *et al.*, 1996). Such an extrapolation starts with the quantification of the relationship between the daily administered amount of TCDD, its accumulation in the body and the associated toxicity in a particular animal species. Once known this relationship has to be extrapolated to man. In the case of the rat several PBPK models have already described the fate of TCDD, inclusive its interactions with the Ah-receptor, (Leung *et al.*, 1990a,b; Kohn *et al.*, 1993; 1996; Carrier *et al.*, 1995a,b; Andersen *et al.*, 1993; 1997a,b). These models can, however, hardly be extrapolated to man. The reason for this is that the scarce data on the disposition of TCDD in man prevents a proper calibration of the relative large number of unknown parameters of these models. We therefore used another approach for the modeling of the kinetics of TCDD. First we developed a generic model for Ah-receptor dependent P450 induction and implemented this model into a PBPK model for TCDD. In developing this model we avoided the uptake of redundant parameters such as diffusion constant for the liver and the slowly and the richly perfused organs, Hill coefficients and cell lysis constants (see for example Kohn *et al.*, 1993, 1996 and Andersen *et al.*, 1993). Furthermore we reduced the number of model parameters by making a quite basic assumption on the P450 induction process. We assumed that all steps in the pathway between the binding of TCDD to the Ah-receptor and P450 translation are instantaneous. In biological terms this assumption means that TCDD binding to the Ah-receptor binding, TCDD-Ah-receptor binding to DNA and P450 transcription occur at a much faster rate than P450 translation. Regarding the timepath P450 transcription and P450 translation occur in TCDD exposed cells (min-hrs resp. days, see for example Xu and Bresnick, 1990, Pendurthi *et al.*, *et al.*, 1993, Schrenk *et al.*, 1991, 1995, Van Iersel, personal communication) we consider this assumption as justified. Notwithstanding the assumption made the model contained 31 parameters. However, 6 parameters could not be obtained from the literature. These parameters were quantified by fitting the model to experimental data. It was found that two P450 induction parameters, i.e. the dissociation constants for the binding of TCDD to the Ah-receptor and for the binding of the TCDD Ah-receptor complex to XRE could not be identified. Here the way the model was defined provided an outcome. For, given the above mentioned assumption on the P450 induction process the parameter which characterises the efficiency of *de novo* P450 synthesis, i.e. the parameter μ (see Appendix), could be identified in the rat liver.

In conclusion we developed a mechanistically based PBPK model for TCDD in the rat. All the parameters of this model could be identified. This, together with the relative low number of parameters of this model, makes a straightforward extrapolation of the rat model to man possible. The accompanying paper describes this extrapolation as well as the application of the resulting human PBPK model in calculating a safe human exposure level of TCDD.

References

Abraham, K., Krowke, R. and D. Neubert. (1988) Pharmacokinetics and biological activity of 2,3,7,8-tetrachlorodibenzo-p-dioxin. 1. Dose-dependent tissue distribution and induction of hepatic ethoxyresorufin-o-deethylase in rats following a single injection. Arch. Toxicol., 62, 359-368.

Ahlborg, U.G., Kimbrough, R.D. and E.J. Yrjänheikki. (1992) Tolerable Daily Intake of PCDDs and PCDFs. Toxic Substances Journal, 12, no. 2-4, 101-331.

Andersen, M.E., Mills, J.J., Gargas, M.L., Kedderis, L., Birnbaum, L.S., Neubert, D. and W.F. Greenlee. (1993) Modeling receptor-mediated processes with dioxin: Implications for pharmacokinetics and risk assessment. Risk Analysis, 13, 25-36.

Aylward, L.L., Hays, S. M., Karch, N.J. and D.J. Paustenbach. (1996) Relative susceptibility of animals and humans to the cancer hazard posed by 2,3,7,8-tetrachlorodibenzo-p-dioxin using internal measures of dose. Environ. Sci. Technol., 30, 3534-3543.

Berg, M. van den, De Jongh, J., Poiger, H. and J.R. Olson. (1994) The toxicokinetics and metabolism of polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) and their relevance for toxicity. Crit. Rev. Toxicol., 24, 1-74.

Carrier, G., Brunet, R.C. and J. Brodeur. (1995a) Modeling of the toxicokinetics of polychlorinated dibenzo-p-dioxins and dibenzofurans in mammalians, including humans. I. Nonlinear distribution of PCDD/PCDF body burden between liver and adipose tissues. Toxicol. Appl. Pharmacol., 131, 253-266.

Carrier, G., Brunet, R.C. and J. Brodeur. (1995b) Modeling of the toxicokinetics of polychlorinated dibenzo-p-dioxins and dibenzofurans in mammalians, including humans. II. Kinetics of absorption and disposition of PCDDs/PCDFs. Toxicol. Appl. Pharmacol., 131, 267-276.

Derks, H.J.G.M., Berende, P.L.M., Everts, H., Olling, M., Liem, A.K.D. and A.P.J.M. de Jong. (1993) A physiologically based pharmacokinetic model of 2,3,7,8-TCDD in the cow. National Institute of Public Health and the Environment, Report nr. 643810.001.

Gasiewicz, T.A., Geiger, L.E., Rucci, G. and R.A. Neal. (1983) Distribution, excretion and metabolism of 2,3,7,8-tetrachlorodibenzo-p-dioxin in C57Bl/6J, DBA/2J and B6D2F$_1$ mice. Drug Metab. Disp., 11, 397-403.

International Life Sciences Institute. (1994) Physiological parameter values for PBPK models. Washington,, USA.

Kedderis, B.L., Diliberto, J.J., Linko, P. Goldstein, J.A. and L.S. Birnbaum. (1991) Disposition of 2,3,7,8-tetrabromodibenzo-p-dioxin and 2,3,7,8-tetrachlorodibenzo-p-dioxin in the rat: Biliary excretion and induction of cytochrome CYP1A1 and CYP1A2. Toxicol. Appl. Pharmacol., 111, 163-172.

Kociba, R.J., Keeler, P.A., Park, C.N. and P.J. Gehring. (1976) 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD): Result of a 13-week oral toxicity study in rats. Toxicol. Appl. Pharmacol., 35, 553-574.

Kociba, R.J., Keyes, D.G., Beyer, J.E., Carreon, R.M., Wade, C.E., Dittenber, D.A., Kalnins, R.P., Frauson, L.E., Parks, C.N., Barnard, S.D., Hummel, R.A. and C.G. Humiston. (1978) Results of a two year chronic toxicity and oncogenicity study of 2,3,7,8-tetrachlorodibenzo-p-dioxin in rats. Toxicol. Appl. Pharmacol., 46, 279-303.

Kohn, M.C., Lucier, G.W., Clark , G.C., Sewall, C., Tritscher, A.M. and C.J. Portier. (1993) A mechanistic model of effects of dioxin on gene expression in the rat liver. Toxicol. Appl. Pharmacol., 120, 138-154.

Kohn, M.C., Lucier, G.W. and C.J. Portier. (1994) The importance of biological realism in dioxin risk assessment models. Risk Analysis, 14, 993-1000.

Kohn, M.C., Sewall, C.H., Lucier, G.W. and C.J. Portier. (1996) A mechanistic model of effects of dioxin on thyroid hormones in the rat. Toxicol. Appl. Pharmacol., 165, 29-48.

Landers, J.P. and N.J. Bunce. (1991) The *Ah* receptor and the mechanism of dioxin toxicity. Biochem. J., 276, 273-287.

Leung, H.W. , Ku, R.H., Paustenbach, D.J. and M.E. Andersen. (1988) A physiologically based pharmacokinetic model for 2,3,7,8-tetrachlorodibenzo-p-dioxin in C57Bl/6J and DBA/2J mice. Toxicology Letters, 42, 15-28.

Leung, H.W. , Paustenbach, D.J., Murray, F.J. and M.E. Andersen. (1990a) A physiological pharmacokinetic description of the tissue distribution and enzyme inducing properties of 2,3,7,8-tetrachlorodibenzo-p-dioxin in the rat. Toxicol. Appl. Pharmacol., 103, 399-410.

Leung, H.W., Poland, A., Paustenbach, D.J., Murray, F.J. and M.E. Andersen. (1990b) Pharmacokinetics of [^{125}I]-2-iodo-3,7,8-trichlorodibenzo-p-dioxin in mice: Analysis with a physiological modeling approach. Toxicol. Appl. Pharmacol., 103, 411-419.

Lucier, G.W., Tritscher, A., Goldworthy, T., Foley, J., Clark, G., Goldstein, J. and R. Maronpot. (1991) Ovarian hormones enhance 2,3,7,8-tetrachlorodibenzo-p-dioxin mediated increases in cell proliferation and preneoplastic foci in a two-stage model for rat hepatocarcinogeneisis. Cancer Res., 51, 1391-1397.

Molen, G.W. van der, Kooijman, S.A.L.M. and W. Slob. (1996) A generic toxicokinetic model for persistent lipophilic compounds in humans: An application to TCDD. Fund. Appl. Toxicol., 31, 83-94.

Pendurthi, U.R., Okino, S.T. and R.H. Tukey. (1993) Accumulation of the nuclear dioxin (Ah) receptor and transcriptional activation of the mouse CYP1a1 and Cyp1a2 genes. Arch. Biochem. Biophys., 306, 65-69.

Poland, A., Teitelbaum, P. and E. Glover. (1989) [^{125}]2-iodo-3,7,8-trichlorodibenzo-p-dioxin binding species in mouse liver induced by agonists for the Ah-receptor: Characterisation and identification. Molec. Pharmacol., 36, 113-120.

Schrenk, D. Lipp, H.P., Wiesmüller, T., Hagenmayer, H. and K.W. Bock. (1991) Assessment of biological activities of mixtures of polychlorinated dibenzo-p-dioxins: Comparison between defined mixtures and their constituents. Arch. Toxicol., 65, 114-118.

Schrenk, D., Stüven, T., Gohl, G., Viebahn, R. and K.W. Bock. (1995) Induction of CYP1A and gluthatione S-transfease activities by 2,3,7,8-tetrachlorodibenzo-p-dioxin in human hepatocyte cultures. Carcinogenesis, 16, 943-946.

Tritscher, A.M., Goldstein, J.A., Portier, C.J., McCoy, Z. and G.C. Clark. (1992) Dose-response relationships for chronic exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin in a rat tumor promotion model: Quantification and immunolocalisation of CYP1A1 and CY1A2 in the liver. Cancer Res., 52, 3436-3442.

Tai, H.L., McReynolds, J.H., Goldstein, J.A., Eugster, H.P., Sengstag, C., Alworth, W.L. and J.R.Olson. Cytochrome P4501A1 mediates the metabolism of 2,3,7,8-tetrachlorodibenzofuran in the rat and humans. Toxicol. Appl. Pharmacol., 123, 34-42.

Watkins, P.B., Bond, J.S. and P.S. Guzelian. (1987) Degradation of hepatic cytochromes P450. In: Mammalian Cytochromes P450 (Guengerich, F.P., Ed.), CRC Press, Boca Raton, Florida, USA.

Xu, L. and E. Bresnick. (1990) Induction of cytochrome P4501A1 in rat hepatoma cell by polycyclic hydrocarbons and a dioxin. Biochem. Pharmacol., 40, 1399-1403.

Zutphen, F.M., Baumans, V. and A.C. Beynen. (1991) Proefdieren en dierproeven. Bunge, Utrecht, The Netherlands.

Appendix

Model for Ah-receptor dependent P450 induction

Model conventions

The model, describing induction at the cellular level, is defined in terms of concentrations. In modeling Ah-receptor dependent P450 induction the following notation is used L: ligand concentration; A: Ah-receptor concentration; X: concentration of Xenobiotic Responsive Elements (XRE)binding sites; F: concentration of cellular fat; P: concentration of P450 proteins; M: concentration of total of metabolites; $\overline{LA}$ concentration of the ligand-Ah-receptor complex; $\overline{LF}$: concentration of ligand dissolved in cellular fat; $\overline{LAX}$: concentration of the ligand-Ah-receptor-XRE complex; $\overline{LP}$: concentration of the ligand-P450 complex. The subscript "0" refers to the total concentration of a cellular constituent. For example, $P_{0,bas}$ refers to the total concentration of basal P450 enzymes. This concentration of basal P450 enzymes is the sum of the concentration of the ligand-P450 complex $\overline{LP}_{bas}$ and the concentration of the unbound enzymes P_{bas}. Similarly, $P_{o,ind}(t)$ refers to the time dependent total concentration of induced P450 enzymes. The subscript "i" refers to the i^{th} Ah-receptor regulated gene.

General model for Ah-receptor dependent P450 induction

The enzyme machinery participating in the metabolism process has two components: a non-Ah-receptor dependent, basal concentration of P450 proteins and an Ah-receptor regulated concentration of inducible P450 enzymes. Assuming a constant basal level of the i^{th} P450 enzyme, ($P_{0,bas,i}$) the cellular concentration of this particular P450 protein is defined as:

$$P_{0,tot,i}(t) = P_{0,ind,i}(t) + P_{0,bas,i} \qquad \text{(A1)}$$

The actual modeling of the induction mechanism is as follows. In the cell the ligand may be found in either of the following forms: taken up in the cellular fat fraction, bound to the Ah-receptor, bound together with the Ah-receptor to XRE sequences, bound to basal and induced P450 proteins and in its "free" (= non-bound/non-dissolved) form. The rate at which the ligand enters into or is released from the cellular fat compartment is modeled as a diffusion process:

$$\frac{d}{dt}\overline{LF} = K_f\left(L(t) - \overline{LF}(t) / p_f\right) \qquad \text{(A2)}$$

Binding of the ligand to the Ah-receptor is modelled as a saturable process:

$$\frac{d}{dt}\overline{LA} = k_{LA}L(t)\cdot A(t) - k_{-LA}\overline{LA}(t) \tag{A3}$$

Similarly the binding of the ligand-Ah-receptor complex to an XRE sequence of the i^{th} Ah-receptor regulated P450 gene is given by:

$$\frac{d}{dt}\overline{LAX_i} = k_{LAX,i}\overline{LA}(t)\cdot X_i(t) - k_{-LAX,i}\overline{LAX_i}(t) \tag{A4}$$

Both equations (A3) and (A4) apply under the condition of constant total concentration of the Ah-receptor and XRE-sequences:

$$\begin{aligned} A_0 &= A(t) + \overline{LA}(t) + \sum_i \overline{LAX_i}(t) \\ X_{i,0} &= X_i(t) + \overline{LAX_i}(t) \end{aligned} \tag{A5}$$

P450 induction was assumed to be solely the result of *de novo* mRNA synthesis occurring in direct proportion to the concentration of the ligand-Ah-receptor-XRE complex, while mRNA catabolism was modeled as a first-order process:

$$\frac{d}{dt}mRNA_{ind,i} = k_{mRNA,i}\overline{LAX_i}(t) - k_{-mRNA,i}mRNA_{ind,i}(t) \tag{A6}$$

Induced P450 translation was modeled as linearly dependent on the mRNA concentration. P450 catabolism is considered to be a first-order process:

$$\frac{d}{dt}P_{0,ind,i} = k_{P,i}mRNA_{ind,i}(t) - k_{-P,i}P_{0,ind,i}(t) \tag{A7}$$

When no metabolites accumulate in the cell, ligand metabolism by a certain Ah-receptor regulated enzyme P_i may be modeled by the following mechanism:

$$L + P_i \underset{k_{-LP,i}}{\overset{k_{LP,i}}{\rightleftarrows}} \overline{LP_i} \begin{matrix} \xrightarrow{k_{m_i}} M + P_i \\ \xrightarrow[k_{-P,i}]{} L + Q_i \end{matrix}$$

where M stands for metabolites and Q for catabolised protein.

So, the rate at which the concentration of the ligand P450 complex changes is given by:

$$\frac{d}{dt}\left[\overline{LP}_{bas,i}+\overline{LP}_{ind,i}\right]=k_{LP,i}\,L(t)\cdot\left[P_{bas,i}(t)+P_{ind,i}(t)\right] \\ -\left[k_{-LP,i}+k_{m,i}+k_{-P,i}\right]\cdot\left[\overline{LP}_{bas,i}(t)+\overline{LP}_{ind,i}(t)\right] \tag{A8}$$

under the condition that:

$$P_{0,bas,i} = P_{bas,i}(t)+\overline{LP}_{bas,i}(t) \\ P_{0,ind,i}(t) = P_{ind,i}(t)+\overline{LP}_{ind,i}(t) \tag{A9}$$

Given $n_0(t)$, the rate at which the ligand enters the cell, the rate at which the total cellular ligand concentration L_0 changes is:

$$\frac{d}{dt}L_0(t)=n_0(t)-\sum_i k_{m,i}\cdot\left[\overline{LP}_{bas,i}(t)+\overline{LP}_{ind,i}(t)\right] \tag{A10}$$

The mass-balance for the cellular ligand concentration gives the expression from which the free concentration $L(t)$ can be solved:

$$L_0(t)=L(t)+\overline{LF}(t)+\overline{LA}(t)+\sum_i\overline{LAX}_i(t) \\ +\sum_i\overline{LP}_{bas,i}(t)+\sum_i\overline{LP}_{ind,i}(t) \tag{A11}$$

Simplifying the model

The application of the model presented in the foregoing paragraph will be limited. The reason for this lies in the great number of model parameters which cannot directly (by experimental means) or indirectly (estimating parameters by fitting the model to a specific data set) be quantified. We therefore simplify the model (and at the same time reduce the number of its parameters) by making a number of assumptions on the biochemical processes underlying the model. First we assume that the ligand-Ah-receptor complex binds with equal efficiency to XRE sequences which are located at various Ah-receptor regulated P450 genes. Second, we assume that the efficiency with which a particular ligand-Ah-receptor-XRE complex enhances P450 transcription cq. P450 translation is equal for all Ah-receptor regulated P450 genes. Furthermore we assume that, while conserving the mass-balance equations (A5) and (A11) of the model, the uptake of the ligand in the cellular fat fraction, the binding of the ligand to the Ah-receptor and to basal and induced P450 proteins, the binding of the ligand-Ah-receptor complex to XRE sequences and induced mRNA synthesis reach equilibrium instantaneously

upon variations in the total ligand concentration $L_0(t)$. Induced P450 protein synthesis is not considered to be instantenous. This way the model describes the ligand concentration in the cellular fat fraction, the ligand-Ah-receptor concentration, the ligand-Ah-receptor-XRE concentration, the concentration of the ligand-P450 complexes and the concentration of induced mRNA as follows.

The ligand concentration in the cellular fat fraction (A2) reduces to:

$$\overline{LF}(t) = p_{fat} L(t) \tag{A12}$$

with $p_{fat} = K_f / p_f$

The ligand-Ah-receptor XRE binding (A4) becomes:

$$\overline{LAX_i}(t) = \frac{X_{0,i}\,\overline{LA}(t)}{K_{d,LAX} + \overline{LA}(t)} \tag{A13}$$

with $K_{d,LAX} = k_{-LAX} / k_{LAX}$.

When the cellular concentration of XRE ligand binding sites is negligibly small, i.e. when $X_{0,i} << K_{d,LAX}$, the total concentration of the ligand-Ah-receptor complex (A3) is approximated by:

$$\overline{LA_0}(t) = \frac{A_0 L(t)}{K_{d,LA} + L(t)} + \sum_i \frac{X_{0,i}\,\overline{LA}(t)}{K_{d,LAX} + \overline{LA}(t)} \approx \frac{A_o L(t)}{K_{d,LA} + L(t)} = \overline{LA}(t) \tag{A14}$$

with $K_{d,LA} = k_{-LA} / k_{LA}$ and neglecting $\sum \overline{LAX_i}(t)$ in (A5) in comparison with $\overline{LA}(t)$.

Likewise, for the ligand-protein binding one obtains:

$$\overline{LP}_{tot,i}(t) = \frac{P_{0,tot,i} L(t)}{K_{d,LP,i} + L(t)} \tag{A15}$$

with $K_{d,LP,i} = (k_{-LP,i} + k_{-P}) / k_{LP,i}$, the effective dissociation constant of the ligand to the i^{th} P450 protein.

For induced mRNA synthesis one obtains:

$$mRNA_{ind,i}(t) = K_{mRNA}\,\overline{LAX_i}(t) \tag{A16}$$

with $K_{mRNA} = k_{mRNA} / k_{-mRNA}$.

We now strive for writing P450 induction as a function of $L(t)$. This is achieved by substituting (A14) into (A13) and the result into (A16). This results in:

$$mRNA_{ind,i}(t) \approx K_{mRNA}\frac{\overline{LAX}_{i,max}L(t)}{\mu + L(t)} \tag{A17}$$

with:

$$\overline{LAX}_{i,max} = \frac{X_{0,i}A_0}{K_{d,lax} + A_0} \qquad \mu = \frac{K_{d,LA}K_{d,LAX}}{A_0 + K_{d,LAX}} \tag{A18}$$

Note that in (A17) the $\overline{LAX_i}(t)$ concentration of (A16) is expressed as an apparent binding of the "free" ligand (intermediated by the ligand-Ah-receptor complex) to XRE-binding sites. Furthermore, given A_0 and X_0, the term $\overline{LAX}_{i,max}$ refers to the maximum concentration of the i^{th} ligand-Ah-receptor-XRE complex that could possibly be formed, i.e. when $\overline{LA} = A_0$, see (A13). Substituting (A17) into (A7), for a particular P450 protein P_i, gives the expression of the rate of induced protein synthesis as a function of $L(t)$:

$$\frac{d}{dt}P_{0,ind,i} \approx k_P K_{mRNA}\frac{\overline{LAX}_{i,max}L(t-\tau)}{\mu + L(t-\tau)} - k_{-P,i}P_{0,ind,i}(t) \tag{A19}$$

The mass-balance for the cellular ligand concentration (A11) is rewritten into:

$$L_0(t) \approx (1 + p_{fat})L(t) + \frac{A_0L(t)}{K_{d,LA} + L(t)} + \sum_i \frac{P_{0,tot,i}(t)L(t)}{K_{d,LP,i} + L(t)} \tag{A20}$$

Then, the rate at which the cellular ligand concentration changes equation (A10) becomes:

$$\frac{d}{dt}L_0(t) \approx n_0(t) - \sum_i \frac{k_{m,i}P_{0,tot,i}(t)L(t)}{K_{d,LP,i} + L(t)} \tag{A21}$$

where $n_0(t)$ stands for the rate at which the ligand enters the cell and $L(t)$ follows implicitly from (A20).

For each protein the P450 induction model depicted in (A19) only contains three parameters: the maximal protein synthesis rate $k_P K_{mRNA} \overline{LAX}_{i,max}$, the rate constant for protein catabolism $k_{\_P}$ and μ

Application in PBPK modelling

The ultimate goal of the concepts introduced in the two preceding sections is the application in a physiologically based pharmacokinetic (PBPK) model that describes the fate of a ligand which induces Ah-receptor mediated *P*-450 cytochrome activity. In particular, the liver is thought of as the compartment that applies to these concepts, but this is not essential: the concepts can be modelled for any compartment where they are of significant importance.

In a typical PBPK model the ligand concentration would be denoted by "C_l", the subscript "*l*" for *l*iver, where $C_l = A_l / V_l$ is the ratio between the amount of the ligand contained in the liver and its volume. This concentration and, e.g., not the concentration in liver cells alone, is usually an observable during or at the end of an experiment. So, one has to find a relation between the compartment concentration C_l and the total ligand concentration L_0 of a cell together with an expression for the amount of ligands entering a cell equivalent with $n_0(t)$ in (A21).

For practical puposes it will be assumed that all the compartment cells are equivalent, i.e. have the same distribution of ligand, Ah-receptor, etc., and receive the same net amount of ligand that enters the compartment. Suppose further instantaneous equilibrium between the arterioles and venules, i.e., the ligand exchanging vessels, and the compartments interstitial fluid free ligand concentration, $L(t)$, as well as equilibrium between the interstitial fluid and the cells free ligand concentration.

Now,

$$C_l = A_l / V_l = v_b C_{l,b} + v_i C_{l,i} + v_c C_{l,c} \qquad \text{(A22)}$$

where v_b, v_i and v_c are the relative volumina of the subcompartmental blood, interstitial fluid and cell compartments respectively. The concentrations $C_{l,b}$, $C_{l,i}$ and $C_{l,c}$ are the respective total ligand concentrations. Note that following this nomenclature the total cell ligand concentration

$$L_0(t) \equiv C_{l,c} \qquad \text{(A23)}$$

By assumption of equilibrium of free ligand concentrations

$$f_b^e C_{l,b} = f_i^e C_{l,i} = f_c^e C_{l,c} \tag{A24}$$

where the fractions $0 \le f^e \le 1$ denote the "free" ligand fractions. Again note that the cell free ligand concentration is

$$L(t) \equiv f_c^e C_{l,c} \tag{A25}$$

From (A22) and (A24) it follows that

$$C_l = \left(\frac{f_c^e}{f_b^e} v_b + \frac{f_c^e}{f_i^e} v_i + v_c \right) \cdot C_{l,c} = \frac{f_c^e}{F^e} C_{l,c} \tag{A26}$$

where F^u is a weighted harmonic mean of the three free fractions:

$$\frac{1}{F^e} = \frac{v_b}{f_b^e} + \frac{v_i}{f_i^e} + \frac{v_c}{f_c^e} \tag{A27}$$

Substituting the new expression for $L_0(t)$ and $L(t)$ in (A20), one obtains

$$C_{l,c} = (1 + p_{fat}) f_c^e C_{l,c} + \frac{A_0 f_c^e C_{l,c}}{K_{d,LA} + f_c^e C_{l,c}} + \sum_i \frac{P_{0,tot,i} f_c^e C_{l,c}}{K_{d,LP,i} + f_c^e C_{l,c}} \tag{A28}$$

From this equality, dividing both sides by $C_{l,c}$ and substituting the expression $F^e C_l$ for $f_c^e C_{l,c}$ (see (A26)) one arrives at the implicit equation for f_c^e:

$$f_c^e = \left[1 + p_{fat} + \frac{A_0}{K_{d,LA} + F^e C_l} + \sum_i \frac{P_{0,tot,i}}{K_{d,LP,i} + F^e C_l} \right]^{-1} \tag{A29}$$

This equation can be solved iterativily by successive substitution of the nth current value of f_c^e, $f_c^e(n)$, in F^e (see A(27)) and then calculating a new value for f_c^e: $f_c^e(n+1)$.

Equivalent to A(26), one arrives at $C_l = f_b^e / F^e \cdot C_{l,b}$ from wich the partition coefficient

$$P_l = \frac{f_b^e}{F^e} \tag{A30}$$

follows. Note that the coefficient is dependent on C_l, because F^e is so via its dependence on f_c^e (A27) which in turn depends on C_l (A29).

The PBPK equation for the liver compartment is thus:

$$\frac{d}{dt} A_l = Q_l (C_a - C_l / P_l) - \sum_i \frac{V_{l,c} k_{m,i} P_{0,tot,i}(t) f_b^e C_l / P_l}{K_{d,LP,i} + f_b^e C_l / P_l} \tag{A31}$$

where $V_{l,c}$ is the livers cell volume.

Calibration and verification of the rodent PBPK model for TCDD

A five compartment physiologically based kinetic (PBPK) model has been chosen to describe the fate of TCDD in mammals. The compartments are the central blood compartment, and the four peripheral compartments fat, slowly perfused, richly perfused and liver. The uptake of TCDD in the fat, the slowly perfused and the richly perfused organ compartments was modeled as a water-fat partitioning process. In the liver compartment the uptake of TCDD was thought to occur also by binding to the Ah-receptor and to Ah-receptor dependent induced P450 proteins.

The administered dose is assumed to diffuse into the central blood compartment, which has been modeled as an input absorption term, see (A32). In previous studies it was found that the uptake of TCDD by the fat compartment could only be modeled as a diffusion limited instead of perfusion limited process (Derks et al., 1993). The kinetics of the fat compartment was therefore assumed to be diffusion limited. This was achieved by subdividing this compartment into a "blood" (10%) and a "tissue" compartment (90%) and modeling transport between these two by a diffusive term, see (A32,A33,A34). The uptake of TCDD in the liver was divided into the uptake of TCDD in the lipid compartment of the liver and in the binding of TCDD to the Ah-receptor and to Ah-receptor dependent TCDD metabolising P450 proteins.

In the rodent liver the proteins 1A1 and 1A2 are induced with almost equal efficiency by TCDD (Tritscher et al., 1992). However, only the induction of the 1A2 protein was found to correlate with the accumulation of TCDD in the liver (Poland et al., 1989). The 1A2 protein therefore was considered as the most important protein binding site for TCDD in the liver. It

was further assumed that neither 1A1 nor 1A2 significantly contributes to the metabolism of TCDD. For, if TCDD metabolism would scale in accordance to these two enzymes a large decrease in the half-life of this compound is expected in animals showing a high induction status of these proteins. Such a decrease however is not observed. To the contrary, in animals showing a substantial increase P450 protein levels TCDD excretion is only induced two-fold (see for example Abraham et al., 1988; Leung et al., 1990). Therefore, TCDD metabolism was modeled to occur by an (unknown) Ah-receptor dependent P450 protein having far higher metabolic but far less dispositional potency than 1A1 and, especially, 1A2. Induction was modeled straightforwardly from the foregoing theory.

This way the following equations were considered to model the data adequately:

$$\frac{d}{dt}A_b = -Q_f(C_b - C_{f,b}) - Q_s(C_b - C_s / P_s) - Q_r(C_b - C_r / P_r) - Q_l(C_b - C_l / P_l) + K_a D_0 e^{-K_a t} \quad \text{(A32)}$$

$$\frac{d}{dt}A_{f,b} = Q_f(C_b - C_{f,b}) - \kappa(f_b^u C_{f,b} - C_{f,t} / P_{f,t}) \quad \text{(A33)}$$

$$\frac{d}{dt}A_{f,t} = \kappa(f_b^u C_{f,b} - C_{f,t} / P_{f,t}) \quad \text{(A34)}$$

$$\frac{d}{dt}A_s = Q_s(C_b - C_s / P_s) \quad \text{(A35)}$$

$$\frac{d}{dt}A_r = Q_r(C_b - C_r / P_r) \quad \text{(A36)}$$

$$\frac{d}{dt}A_l = Q_l(C_b - C_l / P_l) - \sum_{i=1} \frac{k_{m,i} V_{l,c} P_{0,tot,i} f_b^e C_l / P_l}{K_{d,LP,i} + f_b^e C_l / P_l} \quad \text{(A37)}$$

Here, P_l follows from (A30) and $P_{0,tot,i}$'s follow from (A1) and (A19) with $f_b^u C_l / P_l$ substituted for $L(t)$.

The cellular Ethoxyresorufin-O-dealkylase activity (EROD) was modeled as a linear function of the cellular 1A1 and 1A2 concentrations. In this the relative contributions of the 1A1 and 1A2 enzymes to the EROD activity were linearly scaled towards each other (γ=0.05, Tai *et al.*, 1993). Hence :

$$EROD = \beta(P_{0,tot,1} + \gamma \cdot P_{0,tot,2}) \quad \text{(A38)}$$

Model parameter values of the rodent PBPK model

The physiological constants were obtained from Physiological Parameter values for PBPK models as prepared by the International Life Sciences Institute (ILSI), Risk Science Institute, (1994). The absorption coefficient for the oral route was obtained from Leung et al. (1990) and the absorption coefficient for the subcutanous route from Abraham et al. (1988). The coefficients for the partitioning of TCDD between the liver, the Vessel Rich Tissue group and the Muscle Group and the blood plasma were scaled in accordance with their fat percentage (Van der Molen et al., 1996). The parameters characterising TCDD metabolism were chosen such i) that TCDD metabolism followed first-order kinetics with a the terminal half-life in the body of around 18 days and ii) that TCDD metabolism was inducible only two-fold. This value is in concordance with the estimated terminal half-life of TCDD in the rat liver (13-17 days, Abraham et al., 1988) and in the rat adipose tissue (24.5 days, Abraham et al., 1988). The binding constant of TCDD to P450 enzymes other than 1A2 was set equal to the binding constant of TCDD to microsomal binding sites as found in the rodent liver (Poland et al., 1988). The concentration of the cellular Ah-receptor was obtained from Kohn et al. (1994).

The maximal TCDD inducible P450 synthesis rates in rat liver were calculated as follows. When $L(t)$ is in a "steady state", i.e. $L(t) = L_{ss}$, the rate by which a particular P450 protein is synthesized is constant. From (A19) it then follows that the (net) synthesis rate of a particular P450 protein will become zero and so the concentration of the induced P450 protein will reach a "steady state". This concentration is given by:

$$P_{0,ind,i,ss} \approx \frac{v_{ind,max,i}}{k_{-P,i}} \frac{L_{ss}}{\mu + L_{ss}} \qquad \text{(A39)}$$

with maximum $P_{0,ind,\max,i,ss}$ when L_{ss} approaches infinity. The maximal inducible P450 enzyme synthesis rate in the cell then follows from (A39) and is defined as:

$$v_{ind,max,i} \approx P_{0,ind,\max,i,ss} k_{-P,i} \qquad \text{(A40)}$$

By correcting the rate defined by (A40) for the fraction of P450 synthesizing cells in a particular organ one obtains the maximal inducible P450 enzyme synthesis rate for that organ.

An estimate of $P_{0,ind,max,ss}$ may be obtained from experimental studies in which L_{ss} approaches infinity. This situation is approximated in studies in which animals are chronically dosed with high enough doses of TCDD. An example of such a study is the study of Tritscher *et al.* (1992). In this study rats received biweekly TCDD dosed ranging from 50 to 1750 ng/kg for

a period of 30 weeks. We calculated maximal inducible concentrations of 5320 nM and 4330 nM for 1A1 and 1A2 at the end of this period. The basal concentrations of these proteins amounted 22 nM and 535 nM. Given a half-life for P450 proteins of 20 hr in the rat liver (Watkins *et al.*, 1987, $k_{-P,i} = 0.035$ hr^{-1}) the maximal inducible synthesis rates for 1A1 and 1A2 were calculated as 186 nM hr^{-1} and 152 nM hr^{-1} for the rat liver.

The fraction of exchangable TCDD in blood and the interstitial space was assumed to be 1. The relative contribution of the P450 1A2 enzyme to hepatic EROD activity (γ) was obtained from Tai et al. (1993).

The partition coefficient for the fat, the binding constant for TCDD to the hepatic Ah-receptor and of the ligand-Ah-receptor complex to XRE, the binding constant for TCDD to P450 1A2 and the factor relating the P450 1A1 and P450 1A2 concentrations to hepatic EROD activity (β) were obtained by fitting the model to suitable experimental data.

Partition Coefficients

The total concentration in compartment 'x' is a weighted sum over the subcompartmental concentrations

$$C_x = v_{x,p}C_{x,p} + v_{x,i}C_{x,i} + v_{x,c}C_{x,c}$$

where the v's represent the relative volumina of the plasma, interstitial and cellular subcompartments respectively, and the C's the subcompartmental concentrations relative to the subcompartmental volume (and not the total compatment volume). Because of the assumption of well-stirredness, the veneous concentration leaving the compartment is $C_{x,p}$. As in the PBPK model only total compartmental amounts and concentrations are considered, the aim is to express the veneous concentration in terms of the total concentration C_x. It will be assumed throughout that there is no distinction between the plasma and interstitial subcompartment, so $C_x = v_{x,p}C_{x,p} + v_{x,c}C_{x,c}$ and $v_{x,p}$ is to be interpreted as the sum of the relative volumes of the plasma and interstitial subcompartment.

In the following, each subcompartment is subdivided in three phases: water, lipid and protein. So, e.g.,

$$C_{x,p} = v_{x,p,w}C_{x,p,w} + v_{x,p,f}C_{x,p,f} + v_{x,p,p}C_{x,p,p}$$

where the v's represent the relative volumina of the water, lipid and protein phase respectively in the compartments plasma subcompartment, and the C's the concentrations in each phase relative to the phase volume (and not the total subcompartmental volume).

It will be assumed throughout that in all subcompartments protein binding is negligeable $C_{x,y,p} = 0$ for all compartments 'x' and subcompartments 'y'. This would be an absurd approach for the liver compartment, however this comparment is considered separate from the others, i.e. given the approach outlined below, protein binding in the cellular comparment of the liver is 'added' as outlined before.

Furthermore, it will be assumed that equilibrium between the concentrations in the water and lipid phase of each subcompartment of each compartment can be described by only one coefficient, i.e. the octanol-water partition coefficient. Thus, e.g.,

$$C_{x,c} = v_{x,c,w}C_{x,c,w} + v_{x,c,f}C_{x,c,f} = \left(v_{x,c,w} + v_{x,c,f}K_{ow}\right)\cdot C_{x,c,w}$$

Two modeling approaches are developed. In the first approach, it is assumed that the concentrations in the subcompartmental water phases will be instantaneously in equilibrium. In the second approach, it is assumed that the subcompartmentals free exchangeable concentrations are at instanteneous equilibrium. The last approach is quite formal and less understandable than the first one, however, for the fat compartment it leads to partition coefficients much higher than based on the first approach. Such high partition coefficients were reported for the uptake of TCDD in the adipose tissue of experimental animals (Leung *et al.*, 1988; 1992; Andersen *et al.*, 1993; Kohn *et al.*, 1993; 1996). In our PBPK model too, such a high partition coefficient should be taken to account for both the data of liver and fat compartment.

First approach

Equilibration of the concentration in the water phases of the plasma, i.e. plasma + interstitial, and the cellular subcompartment respectively leads to

$$\begin{aligned} C_x &= v_{x,p}C_{x,p} + v_{x,c}C_{x,c} \\ &= v_{x,p}\left(v_{p,w} + v_{p,f}K_{ow}\right)\cdot C_{x,p,w} + v_{x,c}\left(v_{x,c,w} + v_{x,c,f}K_{ow}\right)\cdot C_{x,c,w} \\ &= \left[v_{x,p}\left(v_{p,w} + v_{p,f}K_{ow}\right) + v_{x,c}\left(v_{x,c,w} + v_{x,c,f}K_{ow}\right)\right]\cdot C_{x,p,w} \end{aligned}$$

Hence,

$$\begin{aligned} Q_x &= C_x / C_{x,p} \\ &= \frac{\left[v_{x,p}\left(v_{p,w} + v_{p,f}K_{ow}\right) + v_{x,c}\left(v_{x,c,w} + v_{x,c,f}K_{ow}\right)\right] \cdot C_{x,p,w}}{\left(v_{p,w} + v_{p,f}K_{ow}\right) \cdot C_{x,p,w}} \\ &= v_{x,p} + \frac{v_{x,c,w} + v_{x,c,f}K_{ow}}{v_{p,w} + v_{p,f}K_{ow}} \cdot v_{x,c} \end{aligned}$$

When the octanol-water partition by far exceeds the ratio $v_{p,w} / v_{p,f}$, then it exceeds the ratio $v_{x,c,w} / v_{x,c,f}$ for every compartment, and the partition coefficient can be approximated by

$$Q_x = v_{x,p} + \frac{v_{x,c,f}}{v_{p,f}} \cdot v_{x,c}$$

In practice, the first term can be neglected, and the partition coefficient is proportional to the ratio of the compartments cellular and the plasma relative lipid volume:

$$Q_x = \frac{v_{x,c,f}}{v_{p,f}} \cdot v_{x,c}$$

Second approach

In the second approach it is assumed that there is instantaneous equilibrium between the "free", or perhaps better "exchangeable", concentration fractions of the plasma and cellular subcompartments: $f_p^u C_{x,p} = f_{x,c}^u C_{x,c}$. Then,

$$P_x = C_x / C_{x,p} = v_{x,p} + \frac{C_{x,c}}{C_{x,p}} \cdot v_{x,c} = v_{x,p} + \frac{f_p^u}{f_{x,c}^u} \cdot v_{x,c}$$

When the solution in the water phase is assumed to be free, then the free concentration fraction is

$$f_p^u = \frac{v_{p,w}C_{p,w}}{v_{p,w}C_{p,w} + v_{p,f}C_{p,f}} \qquad f_{x,c}^u = \frac{v_{x,c,w}C_{x,c,w}}{v_{x,c,w}C_{x,c,w} + v_{x,c,f}C_{x,c,f}}$$

for plasma and the cellular subcompartment. Substituting the relation between concentrations in the water and lipid phase respectively, one obtains for the partition coefficient

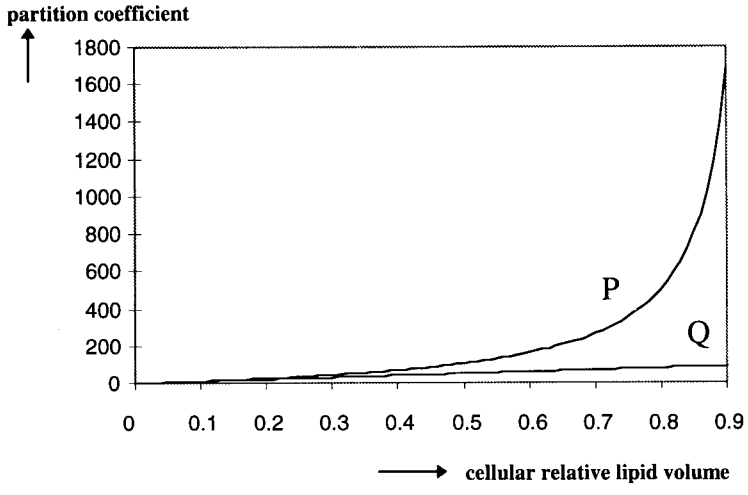
$$P_x = v_{x,p} + \frac{v_{p,w}}{v_{x,c,w}} \cdot \frac{v_{x,c,w} + v_{x,c,f} K_{ow}}{v_{p,w} + v_{p,f} K_{ow}} \cdot v_{x,c}$$

Under the same conditions as above, the partition coefficient is well approximated by

$$P_x = \frac{v_{p,w}}{v_{x,c,w}} \cdot \frac{v_{x,c,f}}{v_{p,f}} \cdot v_{x,c}$$

Note that the partition coefficients P in the second approach are just the coefficients Q multiplied by the ratio of the relative water fractions in the plasma and cellular subcompartment respectively. As the relative water fraction of fat cells is small, this could explain the high values found in the literature, as opposed to values imposed by the first approach. For a comparison see the figure below.

Partition coefficient versus lipid fraction



List of parameter values of the rodent PBPK model

Model parameter	Source	Value (range)
Cardiac output (l/hr, rat of 400 g)	ILSI (1994)	6.6
Tissue Volumes (fraction of body weight)	ILSI (1994)	Liver 0.038 Fat 0.076 VRG[1] 0.019 MG[1] 0.789 Blood 0.078
Tissue perfusion (fraction of cardiac output)	ILSI (1994)	Liver 0.198 Fat 0.076 VRG 0.231 MG 0.495
Absorption coefficient (hr^{-1})	oral : Leung *et al.* (1990) subcutanous : Abraham et al. (1988)	0.2 0.04
Fat-water partition coefficients	Van der Molen *et al.* (1996) (part. coeff. for the fat: calibration)	Liver 5 VRG 5 MG 12.5 Fat 800
Binding constant for TCDD on the AhR ($K_{d,LA}$,nM).	Identifiable in relation to $K_{d,LAX}$ and A_0	see paragraph 3.1
Binding constant for the TCDD-AhR complex on XRE ($K_{d,LAX}$, nM)	Identifiable in relation to $K_{d,LA}$ and A_0	see paragraph 3.1
Binding constant for TCDD on to P450 other than P450 1A2 ($K_{M,LP,i}$, nM)	Poland *et al*, (1988)	56
Binding constant for TCDD on P450 1A2 ($K_{M,LP,1A2}$, nM)	Calibrated	1
Cellular concentration of AhR (A_0, nM)	Kohn *et al.* (1993)	6
Basal hepatic concentration of P450 1A1 ($P_{0,bas,1A1}$, nM)	Tritscher *et al.*, (1992)	25
Basal hepatic concentration of P450 1A2 ($P_{0,bas,1A2}$, nM)	Tritscher *et al.*, (1992)	535

[1] VRG : Vessel Rich Group; MG : Muscle Group

Maximal inducible rate of hepatic P450 1A1 synthesis ($nM \cdot hr^{-1}$)	Calculated (see Appendix)	186
Maximal inducible rate of hepatic P450 1A2 synthesis ($nM \cdot hr^{-1}$)	Calculated (see Appendix)	152
Rate constant for P450 turn over (k_P, hr^{-1})	Guengerich, (1987)	0.035
Diffusion constant for the blood => fat transport (κ, $l \cdot hr^{-1}$)	Calibrated	0.20
Relative contribution of P450 1A2 to EROD activity (γ)	Tai *et al.*, (1993)	0.026
Factor relating the P450 1A1 and 1A2 concentrations to hepatic EROD activity (β)	Calibrated	0.06