— Regular Article —

Human Blood Concentrations of Dichlorodiphenyltrichloroethane (DDT) Extrapolated from Metabolism in Rats and Humans and Physiologically Based Pharmacokinetic Modeling

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The present study defined a simplified physiologically based pharmacokinetic (PBPK) model for dichlorodiphenyltrichloroethane [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane, DDT] in humans based on metabolic parameters determined *in vitro* using relevant liver microsomes, coefficients derived *in silico*, physiological parameters derived from the literature, and an established rat PBPK model. The model consists of an absorption compartment, a metabolizing liver compartment, and a central compartment for DDT. Evaluation of the rat model was performed by making comparisons between predicted concentrations in blood and *in vivo* experimental pharmacokinetic values obtained from rats after daily oral treatment with DDT (10 mg/kg, a no-observed-adverse-effect level) for 14 days. Elimination rates of DDT *in vitro* were established from data from rat liver microsomes and from pooled human liver microsomes. The ratio of intrinsic clearance values of DDT based on rat *in vivo* and rat *in vitro* experiments was used as the scaling factor for estimating *in vivo* hepatic intrinsic clearance in humans in the final human PBPK model. These results indicate that a simplified PBPK model for DDT is useful for a forward dosimetry approach in rats and/or humans and for estimating blood concentrations of other related compounds resulting from exposure to low chemical doses.

Key words —— physiologically based biokinetic modeling, cytochrome P450, simulation, no-observed-adverse-effect level, biomonitoring, human liver microsomes

INTRODUCTION

Appropriate use of human biomonitoring information should be made in risk assessments when creating public policy.[1, 2)] To interpret biomonitoring results, it is of global interest to develop more advanced and accurate risk assessment and risk-based decision making systems.[3–5)] It has been generally attempted to collect extensive information regarding specific physiologically based pharmacokinetic (PBPK) models including pharmacokinetic and/or toxicokinetic parameters found in the literature for predicting concentrations in various biological fluids following multiple dose exposures.[5, 6)] However, although simple, inexpensive, and reliable methods are needed for evaluating the accurate toxic risk, very few have been established.[7)]

Dichlorodiphenyltrichloroethane (1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane, DDT) has been widely used or is still being used as a broad-spectrum insecticide for endemic vector and malaria control and as a treatment for body lice.[8, 9)] DDT is converted in the body and the environment to other more stable chemical forms, including 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (DDE), which are highly persistent in soil, air, and water, as well as in plant and animal tissues.[10–12)]

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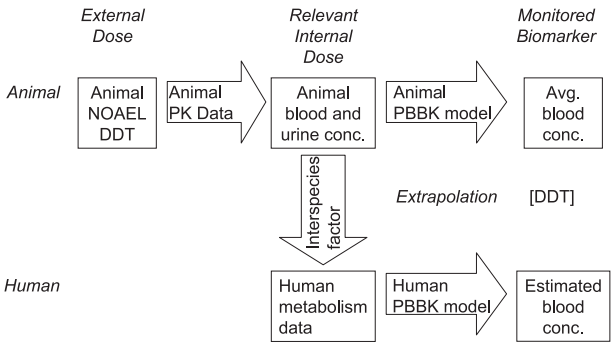

Fig. 1. Approach for Calculating Blood-based Biomonitoring Equivalents for DDT
PK, pharmacokinetics.

Food, particularly meat, fish, milk, and dairy products, continues to be the primary source of DDT exposure, although DDT and DDE intakes have decreased over time.[12, 13] DDT and DDE are distributed to all body tissues but only a small proportion of DDT is metabolized and excreted.[14] The human health effects of DDT at low environmental doses or at biomonitored levels from low environmental exposures are unknown (http://www.cdc.gov/exposurereport/), despite its suggested role as a cause of oxidative stress.[15] Experimental human dosing studies conducted over an 18 month period, during which doses well above environmental levels were given, did not demonstrate overt clinical abnormalities (http://www.atsdr.cdc.gov/substances/index.asp). Multiple reviews of cancer epidemiologic studies have concluded that a link between DDT and cancer has not been clearly established, although the International Agency for Research on Cancer classifies DDT (*p*,*p*′-DDT) as a possible human carcinogen.[16–19]

The purpose of the present study was to carry out a forward dosimetry approach (shown in Fig. 1) using data from chemical doses administered to animals and from *in vitro* experiments with liver microsomes from animals and humans to predict concentrations in humans. As a test substance, DDT was selected. We report herein that the adjusted animal biomonitoring equivalents after orally administered doses at a no-observed-adverse-effect level (NOAEL) in rat studies were scaled to human biomonitoring equivalents using known species allometric scaling factors and human metabolic data with a simple PBPK model.

MATERIALS AND METHODS

Chemicals, Animals, and Enzyme Preparations —— DDT and DDE were obtained from Wako Pure Chemicals (Osaka, Japan). Male rats (7 weeks old) were treated daily with DDT [10 mg/kg body weight (bw)] *per os* (*p.o.*) for 14 days or intraperitoneal (i.p.) for 3 days, based on a NOAEL dose.[20–23] This study was approved by the experimental animal committee of Showa Pharmaceutical University. Liver microsomes from male rats (7 weeks old) treated with DDT (10 mg/kg) and from untreated controls were prepared as described previously.[24] Microsomal P450 contents were determined spectrally by the established method.[25] Protein concentrations were estimated by using a bicinchoninic acid (BCA) protein assay kit (Pierce, Rockford, IL, U.S.A.). Pooled liver microsomes from humans and recombinant P450 enzymes were obtained from BD Biosciences (Woburn, MA, U.S.A.). Typical P450 substrates, their reaction products, and other reagents used in this study were obtained from sources described previously or were of the highest quality commercially available.[24, 26]

Typical P450-dependent marker oxidation activities were measured in liver microsomes in rats to evaluate enzyme inductions on treatment with DDT. Activities for the *O*-dealkylation of ethoxyresorufin (20 μM, for P450 1A) and pentoxyresorufin (100 μM, P450 2B) and for testosterone 7α-hydroxylation (200 μM, P450 2A), tolbutamide methyl hydroxylation (1000 μM, P450 2C), bufuralol 1′-hydroxylation (20 μM, P450 2D), chlorzoxazone 6-hydroxylation (50 μM, P450 2E), and midazolam 1′- and 4-hydroxylation (100 μM, P450 3A) were assayed according to the described HPLC methods.[24, 27, 28]

DDT and DDE Determinations in Biological Samples from Rats —— DDT and DDE in blood and urine samples from individual rats were extracted with ethanol and hexane. DDT and DDE concentrations in these extracts were measured by an HP6890 gas chromatography/mass spectrometry (GC/MS) system equipped with a DB-5MS column (30 m × 0.25 mm i.d. × 0.25 μm, Agilent Technology, Tokyo, Japan) according to the reported methods[22] with slight modifications. To determine the concentrations, the intensity of the precursor ions for DDT (m/z = 235) and DDE (m/z = 246) with internal standard $^{13}C_{12}$-DDT (m/z = 249) and $^{13}C_{12}$-DDE (m/z = 258) were used.

Table 1. Parameters Used for the Rat PBPK Model of DDT

Parameter	Symbol	Value (± S.E.)	Unit
Octanol-water partition coefficient	log$P$	5.34	
Hepatic intrinsic clearance	$CL_{h,int}$	1.14 ± 0.556	l/h
Liver-plasma concentration ratio	$K_{p,h}$	2.92	—
Renal clearance	CL_r	5.97	μl/h
Plasma unbound fraction	$f_{u,p}$	0.012	—
Ratio of the blood to plasma concentration	R_b	1.00	—
Volume of systemic circulation	V_1	0.938 ± 0.235	l
Hepatic volume	V_h	0.00850[23]	l
Hepatic blood flow rate of systemic circulation to the tissue compartment	Q_h	0.853[23]	l/h
Absorption rate constant	k_a	0.346 ± 0.245	h^{-1}
Fraction absorbed × intestinal availability	F_aF_g	1.00	—
Dose	Dose	2.5	mg

Human Metabolic Study —— Elimination rates of DDT in liver microsomes from humans and rats were measured by the GC/MS system mentioned above and were compared. Briefly, a typical incubation mixture consisted of 100 mM potassium phosphate buffer (pH 7.4), an NADPH-generating system, a substrate (1.0 μM), and liver microsomes (0.50 mg protein/ml) in a final volume of 0.25 ml. Incubations were carried out at 37°C for 30 min. The assay linearity with respect to time and protein concentration and the reproducibility (within < 15%) were confirmed. The incubation was terminated by adding 0.40 ml of ice-cold acetonitrile.

Estimation of DDT Concentrations by PBPK Modeling with Suitable Parameters —— A simplified PBPK model was set up as described previously.[7, 23, 29] Parameter values for the physicochemical properties of compounds ($f_{u,p}$, log$P$, $K_{p,h}$, and R_b) are shown in Table 1. Values of $f_{u,p}$ and log$P$ were obtained by *in silico* estimation using SimCYP and ChemDrawBioUltra software;[30] $K_{p,h}$ was estimated from these two values (Appendix A), and R_b was assumed to be 1.0. Parameter values which represent the physiological properties such as hepatic volumes and blood flow rate in rats or humans were taken from the literature.[23] Experimental plasma concentrations of compounds were analyzed by WinNonlin software (Professional version 5.01) with a one-compartment model and yielded primary k_a and elimination constant (k_{el}) values as pharmacokinetic parameters (abbreviations used are also shown in Table 1). Values of total clearance (CL_{tot}), hepatic clearance (CL_h), $CL_{h,int}$, and V_1 were also calculated from the results of the one-compartment model (Appendix B). Subsequently, final parameter values (k_a, $CL_{h,int}$, and V_1) for the rat PBPK model were calculated using the initial values mentioned above by the user model in WinNonlin and are shown in Table 1. Consequently, the following systems of differential equations were solved to conduct the modeling of concentrations in the rat in each compartment shown in Fig. 2:

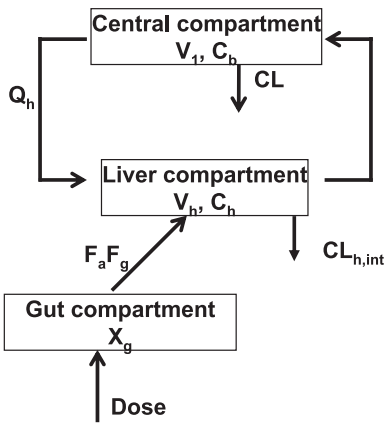

Fig. 2. PBPK Model Established in This Study for Rats and Humans

$$\frac{dX_g(t)}{dt} = -k_a \cdot X_g(t) \quad \text{where at } t = 0,$$

$$X_g(0) = F_a \cdot F_g \cdot Dose,$$

$$V_h \frac{dC_h}{dt} = Q_h \cdot C_b - \frac{Q_h \cdot C_h \cdot R_b}{K_{p,h}} + k_a \cdot X_g - CL_{h,\text{int}} \cdot \frac{C_h}{K_{p,h}} \cdot f_{u,p},$$

$$V_1 \frac{dC_b}{dt} = -Q_h \cdot C_b + \frac{Q_h \cdot C_h \cdot R_b}{K_{p,h}} - CL_r \cdot C_b,$$

where X_g is the substance amount in the gut, C_h is the hepatic substance concentration, and C_b is the blood substance concentration.

Table 2. Parameters Used for the Human PBPK Model

Parameter	Symbol	Value	Unit
Hepatic intrinsic clearance	$CL_{h,int}$	251	l/h
Renal clearance	CL_r	137	μl/h
Volume of systemic circulation	V_1	114	l
Hepatic volume	V_h	1.5[23)]	l
Hepatic blood flow rate systemic circulation to the tissue compartment	Q_h	96.6[23)]	l/h
Absorption rate constant	k_a	0.263	h^{-1}
Dose	Dose	700	mg

Other parameters are the same as those shown in Table 1 for the rat PBPK model.

To define a simplified PBPK model for DDT in humans based on the rat PBPK model, we used relevant liver microsomes and physiological parameters (CL_r, k_a, and V_1) and applied the systems approach to fit them into the traditional parallelogram (animal scale-up strategy) for risk assessment,[5)] as shown in Fig. 1 (Appendix C). The *in vivo* hepatic intrinsic clearance ($CL_{h,int}$) of DDT in humans was estimated by multiplying the calculated initial parameters for *in vitro* hepatic intrinsic clearance in humans by the ratio of *in vivo* to *in vitro* hepatic intrinsic clearance in rats, as mentioned above for modeling in rats. Then, the final parameters for PBPK modeling in humans were calculated and are shown in Table 2. As was done for the rat model, systems of differential equations were solved to obtain concentrations in each compartment in humans.

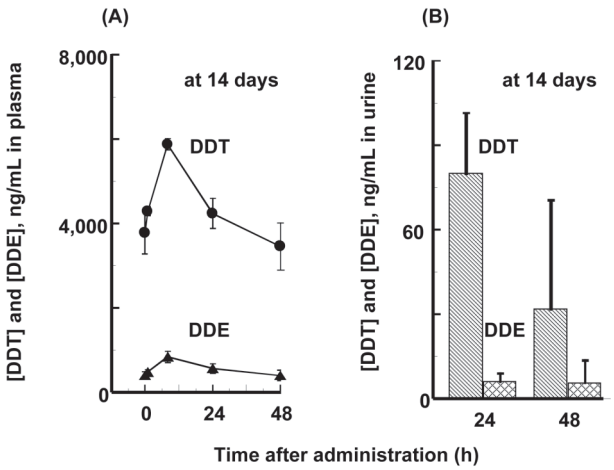

Fig. 3. PK Profiles in Rats Treated with DDT

DDT and DDE concentrations in plasma (A) and urine (B) were determined in rats treated with DDT (10 mg/kg per day) after the final administration of 14 daily doses.

RESULTS

PBPK Model for Rats Orally Treated with DDT

To obtain detailed PBPK model parameters, male rats were orally treated with DDT according to the protocol for general repeated exposure tests. Figure 3 shows the mean levels of DDT and DDE in blood and urine from rats after the final treatment of 14 daily repeated doses of DDT (10 mg/kg). DDT was absorbed and slightly cleared but was apparently accumulated (Fig. 3A). Urinary excretion of DDT and DDE was low within 24 or 48 h after the final repeated administration (Fig. 3B). Renal clearance (CL_r) values of DDT and DDE were calculated from the amounts excreted into the urine (1.01 and 0.104 μg) divided by the area under the curves (1.69×10^5 and 3.24×10^4 μg·h/l), giving 5.97 and 3.21 μl/h, respectively. Primary hepatic clearance values of DDT and DDE were obtained by subtraction of CL_r from the total clearance. Values of the plasma unbound fraction ($f_{u,p}$) of DDT were calculated to be 0.012 , by *in silico* estimation with SimCYP (Table 1).

P450 induction in rat liver microsomes was investigated after intraperitoneal treatment with DDT for 3 days (Fig. 4). Judging from the typical P450-dependent drug oxidation activities, P450 2B- and 2C-mediated activities were slightly increased and decreased, respectively (Fig. 4A), suggesting that P450 induction or suppression by repeated treatments with DDT was almost negligible. Similarly, DDT elimination apparently increased on DDT treatment, but this change was not statistically significant (Fig. 4B). A main role for P450 2B enzymes in DDT elimination was confirmed by immunosuppression with anti-P450 2B antibodies in rat liver microsomes (Fig. 4C); however, several individual P450 enzymes mediated DDT elimination in rats and humans (Fig. 4D).

Consequently, final parameters such as hepatic intrinsic clearance ($CL_{h,int}$), volume of systemic cir-

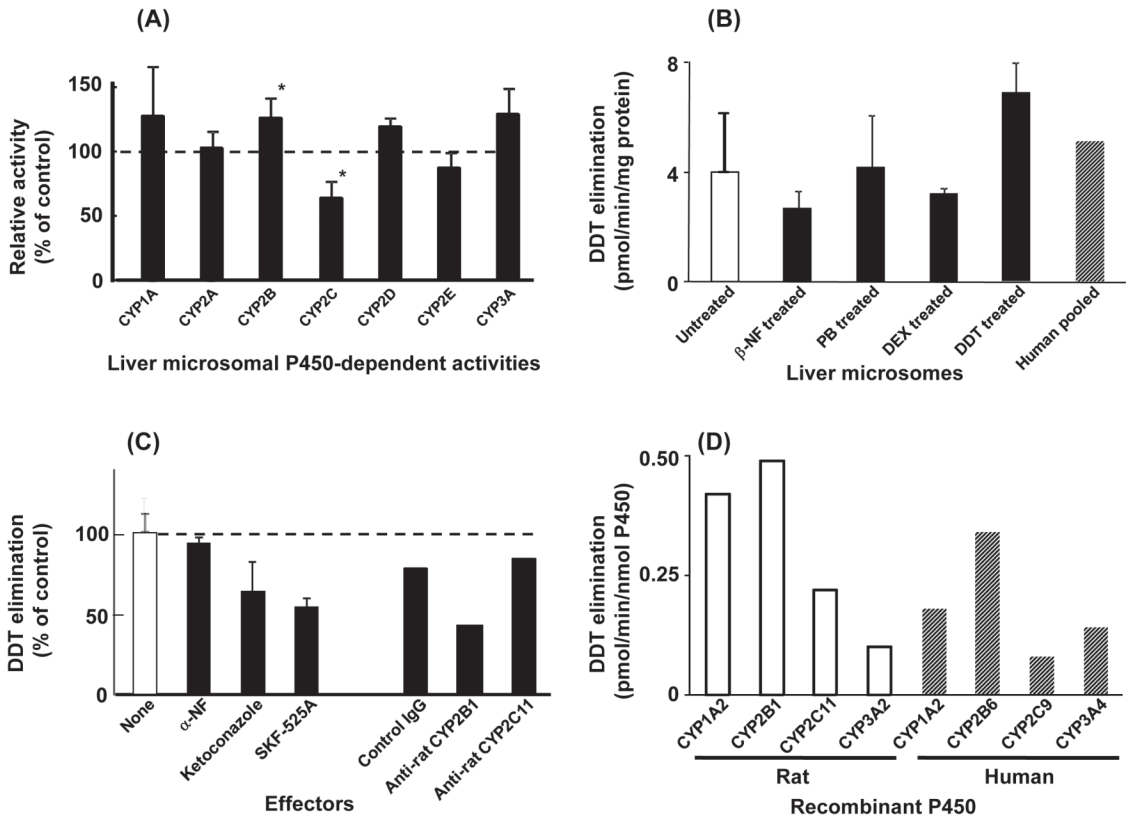

Fig. 4. Roles of P450 Enzymes in DDT Elimination in Liver Microsomes after DDT Treatments

(A) Control activities were taken from liver microsomes from untreated rats. Data columns with bars present means ± S.D.s (n = 4). Significant differences compared with the control activities: $*p < 0.05$. (B and C) P450 2B-dependent DDT elimination rates in liver microsomes induced by pretreatment with DDT. Other rat and human liver microsomes were also used for comparison. Experimental details are shown in Material and Methods. (D) DDT elimination rates catalyzed by recombinant P450 enzymes. β-NF, β-naphthoflavone (a CYP1A inducer); PB, phenobarbital (a CYP2B inducer); DEX, dexamethasone (a CYP3A inducer); and α-NF, naphthoflavone (a CYP1A inhibitor).

culation (V_1), and absorption rate constant (k_a) for the rat PBPK model were recalculated from the primary values by the user model in WinNonlin to give 1.14 l/h, 0.938 l, and 0.346 h^{-1}, respectively, and are shown in Table 1. By running the rat PBPK model system shown in Fig. 2, the blood and liver concentration curves of DDT were estimated after repeated oral administration with 2.5 mg of DDT to a rat (250 g bw); the estimated *in silico* concentration curves of DDT in the liver and blood are shown in Fig. 5A.

Human PBPK Model Supported by *in vitro* Hepatic Clearance Experiments

Hepatic clearance of DDT *in vitro* was determined in pooled human liver microsomes and was compared with data from liver microsomes from rats pretreated with DDT and from untreated controls (Table 3). Hepatic clearance of DDT in human liver microsomes was calculated to be 5.1 μl/min per mg protein; this was similar to the values obtained for rat livers. Subsequently, hepatic intrinsic clearance of DDT was found be 18.4 l/h in an *in vitro* study using the biological coefficients already established. The intrinsic clearance values of DDT based

Table 3. *In vitro* Hepatic Intrinsic Clearance of DDT Determined Using Liver Microsomes

Enzyme source	Clearance	
	μl/min per mg protein	l/h[a)]
Rats, untreated[b)]	4.0 ± 1.8	0.0721
Rats, treated with DDT[b)]	6.9 ± 1.1	0.124
Pooled human livers	5.1	18.4

DDT (1.0 μM) was incubated with rat or human liver microsomes in the presence of an NADPH-generating system. The reduction rates of DDT were determined by GC/MS.

a) Estimated clearance values were extrapolated using the following values: 40 mg liver microsomal protein per g liver, 10 g liver weight per 0.25 kg of rat body weight, and 1.5 kg liver per 70 kg of human body weight. *b*) Mean ± S.D. (n = 4) values using liver microsomes from individual rats pretreated with DDT (10 mg/kg) daily for 3 days or from untreated controls.

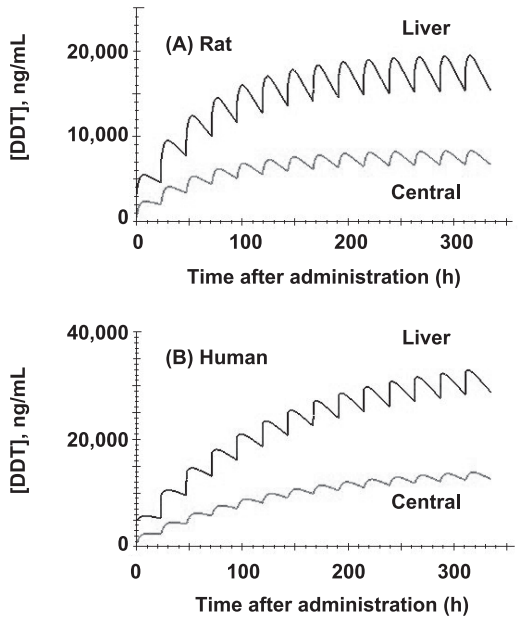

Fig. 5. Estimated Blood (Central) and Liver Concentrations by PBPK Modeling of Daily Oral Administration of DDT (10 mg/kg) for 14 days in Rats (A) and Humans (B)

The curves show the estimated concentrations in the liver and central (blood) compartments of the model.

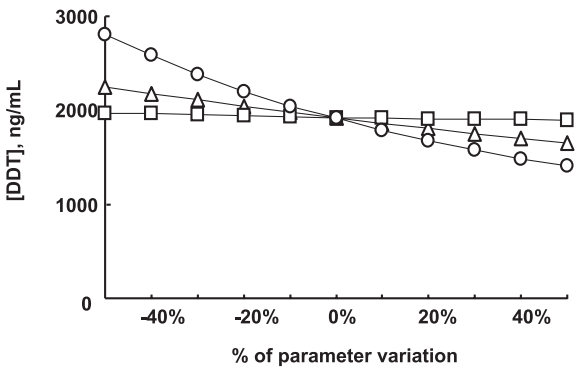

Fig. 6. Sensitivity Analysis of Rat PBPK Modeling of DDT

Blood concentrations of DDT at 24 hr after the oral administration to rats were estimated with increasing and decreasing values of parameters, $CL_{h,\mathrm{int}}$ (triangles), V_1 (circles), and k_a (squares), shown in Table 1 with mean ± S.E. values.

on rat *in vivo* (Table 1) and rat *in vitro* (Table 3) experiments were different; this ratio (1.14/0.124) was used as the compensating factor for estimating *in vivo* hepatic intrinsic clearance in humans. Finally, a value of 251 l/h for the DDT hepatic intrinsic clearance ($CL_{h,\mathrm{int}}$) was adopted to represent the *in vivo* status in the final human PBPK model, the parameters of which are shown in Table 2.

Figure 6 indicates how the variation (uncertainty) in the output of the present rat PBPK model of DDT can be apportioned, qualitatively or quantitatively. Figure 5B indicates the estimated human blood concentrations of DDT after modeling repeated oral administration with DDT (10 mg/kg). The apparent maximum blood concentration of DDT was estimated to be approximately 10000 ng/ml. When daily administration of DDT was modeled for 14 days, some accumulation of DDT in the liver (approximately 30000 ng/ml) was estimated by the present human PBPK model. In separate rat experiments, DDT accumulation was found in the liver at a level of 13000 ± 2500 ng/g (mean ± S.D., $n = 4$) after treatment with 3 daily i.p. injections. This level was well estimated by the present PBPK model at day 4, as shown in Fig. 5A.

DISCUSSION

In general, levels of DDT and DDE in the blood increase as a person ages as a result of cumulative exposure (http://www.atsdr.cdc.gov/substances/). Since the 1970 s, mean serum levels of DDT and DDE in the U.S.A. population have declined about fivefold to tenfold.[31, 32] High mean levels of whole blood DDT (about 3.86 ng/ml) and DDE (about 14.49 ng/ml) were found in a study of pesticide workers in Argentina.[33] Workers involved in production or application of DDT have developed neurologic abnormalities associated with blood levels of around 100000 ng/ml.[16–19] Finding a measurable amount of DDT or DDE in serum does not mean that the level of the chemical will cause adverse health effects.[16–19] Biomonitoring studies on levels of DDT and DDE provide physicians and public health officials with reference values so that they can determine whether people have been exposed to higher levels of DDT or DDE than are found in the general population.

It is generally accepted that PBPK modeling could be of use for understanding the relationship between chemical exposure and concentrations in body fluids (Fig. 1). However, the multiple compartments and many complicated equations found in traditional PBPK modeling cause severe difficulties when applying the model. Simple and reliable methods have not yet been established, but such models are needed to explore the biological significance of a wide range of chemicals. The present study defined a simplified PBPK model for DDT in humans (Fig. 2). Because of the simplicity of our adopted PBPK model, a reliable human hepatic clearance value and a complete set of human

PBPK parameters could be estimated from limited experiments, namely oral dose administration in rats (Fig. 3) and *in vitro* clearance experiments with rat and human liver microsomes (Table 3) in the present study. The developed PBPK model for DDT in rats simply consisted of three compartments, *i.e.*, the gut as the chemical absorption compartment, the liver as the metabolizing compartment, and the general circulation as the central compartment (Fig. 2). In laboratory animals, DDT may induce specific cytochrome P450 enzymes to some extent (Fig. 4), as reported previously,[34] but this information suggests that more complex modeling of the liver compartment is not needed in the simple PBPK model used in this study. Despite the single time point determination, apparent accumulation of DDT in the liver estimated using the simple PBPK model (Fig. 5) was confirmed by the measurement of DDT concentrations in the liver at day 4 after 3 daily treatments of rats with DDT. These results are consistent with a calculated long half life of DDT in the human body.[7] Estimated biomonitoring data can also help scientists plan and conduct research on exposure and health effects.

Human biomonitoring is important for many aspects of environmental health.[1,2] Recently, the Centers for Disease Control and Prevention in the United State reported several pieces of relevant data, including the 95th percentile values of serum DDE levels (approximately 2 ng/ml, http://www.cdc.gov/exposurereport/). Based on our forward dosimetry approach and assuming full conversion from DDT to DDE, the equivalent level of DDT exposure was estimated to be approximately 2 μg/day in the United States.

Evaluation of the developed rat model was performed by comparing the blood concentrations predicted by PBPK modeling *in silico* and experimental pharmacokinetic values from plasma and urine obtained from rats *in vivo* after repeated oral treatment with DDT at a NOAEL. To overcome the species differences in animals and humans, the traditional parallelogram technique[35,36] used in systems biology[4,5] was adapted for this study to estimate the value of *in vivo* human hepatic clearance from *in vitro* data (Table 3).

In summary, the present study indicates that simplified PBPK modeling for DDT is useful for a forward dosimetry approach in rats and humans to estimate blood concentrations of DDT and other related compounds from low chemical doses such as those at the NOAEL.

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Appendix A The liver-plasma concentration ratio ($K_{p,H}$) was calculated from Eq. A1:[37]

$$K_{p,h} = \frac{P \times 0.02289 + 0.72621}{P \times 0.00396 + 0.960581} \times \frac{f_{u,p}}{f_{u,h}} \quad \text{(A1)}$$

where P is the water-octanol partition ratio and was estimated from the computer-calculated $\log P$ as neutral ($c \log P$):

$$P = 10^{\log P} \quad \text{(A2)}$$

$f_{u,h}$ is the hepatic unbound fraction for a specific binding on albumin, globulins, and lipoproteins. The tissue interstitial fluid-to-plasma concentration ratios of albumin, globulins, and lipoproteins were assumed to be 0.5:

$$\frac{f_{u,p}}{f_{u,h}} = 0.5 \times \left(f_{u,p} + 1\right) \quad \text{(A3)}$$

Appendix B The initial parameter values of $CL'_{h,\mathrm{int}}$ and V'_1 used to execute the fitting calculation of the PBPK model with WinNonlin software were derived from the follow equations.

Hepatic clearance (CL_h) was estimated from Eq. B1, which was derived from Eq. B2:

$$CL_h = \frac{Dose \times Q_h - AUC \times CL_r \times Q_h}{AUC \times Q_h + Dose} \quad \text{(B1)}$$

$$\frac{CL_{tot}}{F} = \frac{CL_h + CL_r}{1 - \dfrac{CL_h}{Q_h}} = \frac{Dose}{AUC} \quad \text{(B2)}$$

where AUC is the area under the curve.

The bioavailability (F), fraction absorbed (F_a), and intestinal availability (F_g) are related as:

$$F = F_a \times F_g \times F_h \quad \text{(B3)}$$

where F_h is fraction unmetabolized in the liver.

In this study, we assume $F_a F_g = 1.0$; then, the bioavailability was calculated from Eq. B4 (the prime represents the value under the assumption of $F_a F_g = 1.0$):

$$F' = F_a F_g \times F_h = F_h = 1 - \frac{CL_h}{Q_h} \quad \text{(B4)}$$

The initial value of V_1 was estimated from Eq. B5 using the fitted calculation results of the one-compartment model (V_d/F) and the F' value from

Eq. B4:

$$V_1' = (V_d/F) \times F' \quad \text{(B5)}$$

The initial value of hepatic intrinsic clearance ($CL_{h,\mathrm{int}}$) was estimated from Eq. B6, where CL_h was evaluated from Eqs. B7 and B8:

$$CL_{h,\mathrm{int}}{}' = \frac{R_b}{f_{u,p}} \times \frac{Q_h \times CL_h}{Q_h - CL_h} \quad \text{(B6)}$$

$$CL_{tot} = (V_d/F) \times k_{el} \times F' \quad \text{(B7)}$$

$$CL_h = CL_{tot} - CL_r \quad \text{(B8)}$$

Values of adjusted distribution volume (V_d/F) and elimination constant (k_{el}) were calculated from the fitting calculation of the one-compartment model, and Eq. B7 was derived from Eq. B9:

$$\frac{CL_{tot}}{F} = (V_d/F) \times k_{el} \quad \text{(B9)}$$

The initial value of k_a for the fitting calculation was used as the primary results of WinNonlin with the one-compartment model.

Appendix C The parameter values of CL_r, k_a, and V_1 in the human PBPK model were estimated using a scale-up strategy from rats to humans as follows. Human renal clearance $CL_{r,human}$ was estimated from Eq. C1, which was derived from Eq. C2, where $BW_{rat} = 0.25$ kg and $BW_{human} = 70$ kg:

$$CL_{r,human} = \frac{CL_{r,rat}}{BW_{rat}{}^{\frac{2}{3}}} \times BW_{human}{}^{\frac{2}{3}} \quad \text{(C1)}$$

$$CL_r = a \times BW^{\frac{2}{3}} \quad \text{(C2)}$$

The human systemic circulation volume ($V_{1,human}$) was estimated from Eqs. C3 and C4, where $V_{h,human}$, $V_{b,rat}$, and $V_{b,human}$ were 1.5, 0.016, and 4.9 l, respectively:

$$V_{1,human} = V_{d,human} - V_{h,human} \times \frac{K_{p,h} \times F_h}{R_b} \quad \text{(C3)}$$

$$V_{d,human} = V_{b,human} + (V_{d,rat} - V_{b,rat}) \times \frac{R_{b,rat}}{f_{u,p,rat}} \times \frac{f_{u,p,human}}{R_{b,human}} \quad \text{(C4)}$$

where physicochemical parameters such as $K_{p,h}$, R_b, and $f_{u,p}$ were assumed to be consistent between rats and humans The derivation of $K_{p,h}$ is shown in Appendix A.

The human absorption rate constant (k_a) was estimated from Eq. C5:[38]

$$k_{a,human} = 0.744 \times k_{a,rat} \quad \text{(C5)}$$

REFERENCES

1) Collins, F. S., Gray, G. M. and Bucher, J. R. (2008) Toxicology. Transforming environmental health protection. *Science*, **319**, 906–907.
2) National Research Council of the National Academies (2007) *Toxicity Testing in the 21st Century: A Vision and a Strategy*, The National Academies Press, Washington, D.C.
3) Wild, C. P. (2005) Complementing the genome with an "exposome": the outstanding challenge of environmental exposure measurement in molecular epidemiology. *Cancer Epidemiol. Biomarkers Prev.*, **14**, 1847–1850.
4) Hayes, K. R. and Bradfield, C. A. (2005) Advances in toxicogenomics. *Chem. Res. Toxicol.*, **18**, 403–414.
5) Edwards, S. W. and Preston, R. J. (2008) Systems biology and mode of action based risk assessment. *Toxicol. Sci.*, **106**, 312–318.
6) Clewell, H. J., Gentry, P. R., Covington, T. R., Sarangapani, R. and Teeguarden, J. G. (2004) Evaluation of the potential impact of age- and gender-specific pharmacokinetic differences on tissue dosimetry. *Toxicol. Sci.*, **79**, 381–393.
7) Verner, M. A., Ayotte, P., Muckle, G., Charbonneau, M. and Haddad, S. (2009) A physiologically based pharmacokinetic model for the assessment of infant exposure to persistent organic pollutants in epidemiologic studies. *Environ. Health Perspect.*, **117**, 481–487.
8) Pan, I. J., Daniels, J. L., Goldman, B. D., Herring, A. H., Siega-Riz, A. M. and Rogan, W. J. (2009) Lactational exposure to polychlorinated biphenyls, dichlorodiphenyltrichloroethane, and dichlorodiphenyldichloroethylene and infant neurodevelopment: an analysis of the pregnancy, infection, and nutrition babies study. *Environ. Health Perspect.*, **117**, 488–494.
9) Rivero-Rodriguez, L., Borja-Aburto, V. H., Santos-Burgoa, C., Waliszewskiy, S., Rios, C. and Cruz, V. (1997) Exposure assessment for workers applying DDT to control malaria in Veracruz, Mexico. *Environ. Health Perspect.*, **105**, 98–101.
10) Toppari, J., Larsen, J. C., Christiansen, P., Giwercman, A., Grandjean, P., Guillette, L. J., Jr., Jegou, B., Jensen, T. K., Jouannet, P., Keiding, N., Leffers, H., McLachlan, J. A., Meyer, O., Muller, J., Rajpert-De, M. E., Scheike, T., Sharpe, R., Sumpter, J. and Skakkebaek, N. E. (1996) Male reproductive health and environmental xenoestrogens. *Environ. Health Perspect.*, **104** Suppl. 4, 741–803.
11) Kitamura, S., Shimizu, Y., Shiraga, Y., Yoshida,

M., Sugihara, K. and Ohta, S. (2002) Reductive metabolism of *p*,*p*′-DDT and *o*,*p*′-DDT by rat liver cytochrome P450. *Drug Metab. Dispos.*, **30**, 113–118.
12) Smith, D. (1999) Worldwide trends in DDT levels in human breast milk. *Int. J. Epidemiol.*, **28**, 179–188.
13) Gunderson, E. L. (1988) FDA Total Diet Study, April 1982-April 1984, dietary intakes of pesticides, selected elements, and other chemicals. *J. Assoc. Off. Anal. Chem.*, **71**, 1200–1209.
14) Morgan, D. P. and Roan, C. C. (1971) Absorption, storage, and metabolic conversion of ingested DDT and DDT metabolites in man. *Arch. Environ. Health*, **22**, 301–308.
15) Harada, T., Yamaguchi, S., Ohtsuka, R., Takeda, M., Fujisawa, H., Yoshida, T., Enomoto, A., Chiba, Y., Fukumori, J., Kojima, S., Tomiyama, N., Saka, M., Ozaki, M. and Maita, K. (2003) Mechanisms of promotion and progression of preneoplastic lesions in hepatocarcinogenesis by DDT in F344 rats. *Toxicol. Pathol.*, **31**, 87–98.
16) Eskenazi, B., Chevrier, J., Rosas, L. G., Anderson, H. A., Bornman, M. S., Bouwman, H., Chen, A., Cohn, B. A., de Jager, C., Henshel, D. S., Leipzig, F., Leipzig, J. S., Lorenz, E. C., Snedeker, S. M. and Stapleton, D. (2009) The Pine River statement: human health consequences of DDT use. *Environ. Health Perspect.*, **117**, 1359–1367.
17) Beard, J., Sladden, T., Morgan, G., Berry, G., Brooks, L. and McMichael, A. (2003) Health impacts of pesticide exposure in a cohort of outdoor workers. *Environ. Health Perspect.*, **111**, 724–730.
18) Calle, E. E., Frumkin, H., Henley, S. J., Savitz, D. A. and Thun, M. J. (2002) Organochlorines and breast cancer risk. *CA Cancer J. Clin.*, **52**, 301–309.
19) Snedeker, S. M. (2001) Pesticides and breast cancer risk: a review of DDT, DDE, and dieldrin. *Environ. Health Perspect.*, **109** Suppl. 1, 35–47.
20) Tomiyama, N., Takeda, M., Watanabe, M., Kobayashi, H. and Harada, T. (2004) A further study on the reliability of toxicokinetic parameters for predicting hepatotoxicity in rats receiving a 28-day repeated administration of DDT. *J. Toxicol. Sci.*, **29**, 505–516.
21) Tomiyama, N., Watanabe, M., Takeda, M., Harada, T. and Kobayashi, H. (2003) A comparative study on the reliability of toxicokinetic parameters for predicting hepatotoxicity of DDT in rats receiving a single or repeated administration. *J. Toxicol. Sci.*, **28**, 403–413.
22) Guardino, X., Serra, C., Obiols, J., Rosell, M. G., Berenguer, M. J., Lopez, F. and Brosa, J. (1996) Determination of DDT and related compounds in blood samples from agricultural workers. *J. Chromatogr. A*, **719**, 141–147.
23) You, L., Gazi, E., Archibeque-Engle, S., Casanova, M., Conolly, R. B. and Heck, H. A. (1999) Transplacental and lactational transfer of p,p′-DDE in Sprague-Dawley rats. *Toxicol. Appl. Pharmacol.*, **157**, 134–144.
24) Yamazaki, H., Shimizu, M., Nagashima, T., Minoshima, M. and Murayama, N. (2006) Rat cytochrome P450 2C11 in liver microsomes involved in oxidation of anesthetic agent propofol and deactivated by prior treatment with propofol. *Drug Metab. Dispos.*, **34**, 1803–1905.
25) Omura, T. and Sato, R. (1964) The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. *J. Biol. Chem.*, **239**, 2370–2378.
26) Murayama, N., Kaneko, N., Horiuchi, K., Ohyama, K., Shimizu, M., Ito, K. and Yamazaki, H. (2009) Cytochrome P450-dependent drug oxidation activity of liver microsomes from Microminipigs, a possible new animal model for humans in non-clinical studies. *Drug Metab. Pharmacokinet.*, **24**, 404–408.
27) Yamazaki, H., Mimura, M., Sugahara, C. and Shimada, T. (1994) Catalytic roles of rat and human cytochrome P450 2A enzymes in testosterone 7α- and coumarin 7-hydroxylations. *Biochem. Pharmacol.*, **48**, 1524–1527.
28) Yamazaki, H., Nakamura, M., Komatsu, T., Ohyama, K., Hatanaka, N., Asahi, S., Shimada, N., Guengerich, F. P., Shimada, T., Nakajima, M. and Yokoi, T. (2002) Roles of NADPH-P450 reductase and apo- and holo-cytochrome b_5 on xenobiotic oxidations catalyzed by 12 recombinant human cytochrome P450s expressed in membranes of *Escherichia coli*. *Protein Expr. Purif.*, **24**, 329–337.
29) Kato, M., Shitara, Y., Sato, H., Yoshisue, K., Hirano, M., Ikeda, T. and Sugiyama, Y. (2008) The quantitative prediction of CYP-mediated drug interaction by physiologically based pharmacokinetic modeling. *Pharm. Res.*, **25**, 1891–1901.
30) Emoto, C., Murayama, N., Rostami-Hodjegan, A. and Yamazaki, H. (2009) Utilization of estimated physicochemical properties as an integrated part of predicting hepatic clearance in the early drug-discovery stage: Impact of plasma and microsomal binding. *Xenobiotica*, **39**, 227–235.
31) Stehr-Green, P. A. (1989) Demographic and seasonal influences on human serum pesticide residue levels. *J. Toxicol. Environ. Health*, **27**, 405–421.
32) Anderson, H. A., Falk, C., Hanrahan, L., Olson, J., Burse, V. W., Needham, L., Paschal, D., Patterson, D., Jr. and Hill, R. H., Jr. (1998) Profiles of Great

Lakes critical pollutants: a sentinel analysis of human blood and urine. The Great Lakes Consortium. *Environ. Health Perspect.*, **106**, 279–289.

33) Radomski, J. L., Astolfi, E., Deichmann, W. B. and Rey, A. A. (1971) Blood levels of organochlorine pesticides in Argentina: occupationally and nonoccupationally exposed adults, children and newborn infants. *Toxicol. Appl. Pharmacol.*, **20**, 186–193.
34) Nims, R. W., Lubet, R. A., Fox, S. D., Jones, C. R., Thomas, P. E., Reddy, A. B. and Kocarek, T. A. (1998) Comparative pharmacodynamics of CYP2B induction by DDT, DDE, and DDD in male rat liver and cultured rat hepatocytes. *J. Toxicol. Environ. Health A*, **53**, 455–477.
35) Takano, R., Murayama, N., Horiuchi, K., Kitajima, M., Kumamoto, M., Shono, F. and Yamazaki, H. Blood concentrations of acrylonitrile in humans after oral administration extrapolated from *in vivo* rat pharmacokinetics, *in vitro* human metabolism, and physiologically based pharmacokinetic modeling. *Regul. Toxicol. Pharmacol.*, in press.
36) Takano, R., Murayama, N., Horiuchi, K., Kitajima, M., Shono, F. and Yamazaki, H. (2010) Blood concentrations of 1,4-dioxane in humans after oral administration extrapolated from *in vivo* rat pharmacokinetics, *in vitro* human metabolism, and physiologically based pharmacokinetic modeling. *J. Health Sci.*, **56**, 557–565.
37) Poulin, P. and Theil, F. P. (2002) Prediction of pharmacokinetics prior to in vivo studies. 1. Mechanism-based prediction of volume of distribution. *J. Pharm. Sci.*, **91**, 129–156.
38) Amidon, G. L., Sinko, P. J. and Fleisher, D. (1988) Estimating human oral fraction dose absorbed: a correlation using rat intestinal membrane permeability for passive and carrier-mediated compounds. *Pharm. Res.*, **5**, 651–654.