— **Regular Article** —

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

Ryohji Takano,[a, b] Norie Murayama,[a] Kana Horiuchi,[a] Masato Kitajima,[b] Fumiaki Shono,[c] and Hiroshi Yamazaki*[, a, d]

[a]Laboratory of Drug Metabolism and Pharmacokinetics, Showa Pharmaceutical University, 3–3165 Higashi-tamagawa Gakuen, Machida, Tokyo 194–8543, [b]Fujitsu Kyusyu Systems, Momochihama, Sawara-ku, Fukuoka 814–8589, [c]Japan Chemical Industry Association (JCIA), 1–4–1 Shinkawa, Chuo-ku, Tokyo 104–0033, Japan and [d]High Technology Research Center, Showa Pharmaceutical University, 3–3165 Higashi-tamagawa, Gakuen, Machida, Tokyo 194–8543, Japan

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The present study defined a simplified physiologically based pharmacokinetic (PBPK) model for 1,4-dioxane in humans based on *in vitro* metabolic parameters determined using relevant liver microsomes, coefficients derived *in silico*, physiological parameters derived from the literature, and a developed PBPK model in rats. The model consists of a chemical absorption compartment, a metabolizing compartment, and a central compartment for 1,4-dioxane. Evaluation of the rat model was performed by comparisons with experimental pharmacokinetic values from blood and urine obtained from rats *in vivo* after daily oral treatment with 1,4-dioxane (500 mg/kg, a no-observed-adverse-effect level) for 14 days. Elimination rates of 1,4-dioxane *in vitro* were established using data from rat liver microsomes and from pooled human liver microsomes. 1,4-Dioxane was expected to be absorbed and cleared rapidly from the body *in silico*, as was the case for rats confirmed experimentally *in vivo* with repeated low-dose treatments. These results indicate that the simplified PBPK model for 1,4-dioxane is useful for a forward dosimetry approach in humans. This model may also be useful for simulating 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

Basic information is necessary to interpret human biomonitoring results obtained internationally to promote risk-based decision making.[1–3] Simplified, advanced, and accurate risk assessment systems are of global interest to support appropriate interpretation and communication based on human biomonitoring results.[4] Pharmacokinetic and/or toxicokinetic parameters for a variety of chemicals have been determined in animal toxicology studies, even when limited corresponding data exist for humans.[5] Species differences of drug-metabolizing enzymes in the liver, including cytochrome P450 (P450 or CYP) enzymes, are the focus for understanding qualitative and quantitative differences in concentrations in biological fluids or chemical exposures in animals and in humans.[6] It has been generally attempted to collect extensive information regarding specific physiologically based pharmacokinetic (PBPK) models found in the literature for predicting concentrations in various biological fluids following multiple dose exposures.[2] However, although simple, inexpensive, and reliable methods are needed for evaluating the accurate toxic risk, only very complicated models have so far been established.[7, 8]

*To whom correspondence should be addressed: Laboratory of Drug Metabolism and Pharmacokinetics, Showa Pharmaceutical University, 3–3165 Higashi-tamagawa Gakuen, Machida, Tokyo 194–8543, Japan. Tel. & Fax: +81-42-721-1406; E-mail: hyamazak@ac.shoyaku.ac.jp

1,4-Dioxane (Fig. 1) is widely used, primarily as a solvent or as a solvent stabilizer,[9] but subchronic oral toxicity of 1,4-dioxane has been examined in rats by administering 1,4-dioxane in drinking water, suggesting hepatic and renal failures.[10] 1,4-Dioxane is believed to be primarily metabolized by P450s to 2-(2-hydroxyethoxy)acetic acid or its interconversion product 1,4-dioxiane-2-one (Fig. 1).[7,8] Kinetic models for 1,4-dioxane and its major metabolite (hydroxyethoxyacetic acid) were published in 1990;[11,12] however, the uncertainties and deficiencies of these models for use in a contemporary cancer risk assessment for 1,4-dioxane have been pointed out.[8]

Therefore, the purpose of the present study was to carry out a forward dosimetry approach (shown in Fig. 2) using data from chemical doses administered to animals and from *in vitro* experiments with liver microsomes from humans and animals to predict 1,4-dioxane concentrations in humans. 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.

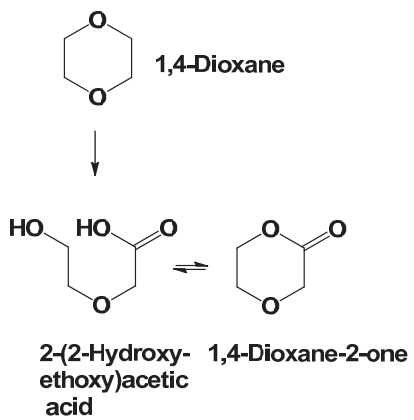

Fig. 1. Chemical Structures of 1,4-dioxane and Reported Metabolites

MATERIALS AND METHODS

Chemicals, Animals, and Enzyme Preparations —— 1,4-Dioxane was obtained from Wako Pure Chemicals (Osaka, Japan). Male Sprague-Dawley rats (7 weeks old, 180 g) were treated daily with 1,4-dioxane [500 mg/kg body weight (bw)] *per os* (*p.o.*) for 14 days, based on a NOAEL dose.[8] This study was approved by the experimental animal committee of Showa Pharmaceutical University. In separated experiments, liver microsomes from male Sprague-Dawley rats (7 weeks old, 180 g) intraperitoneal (i.p). treated with 1,4-dioxane (500 mg/kg) according to typical P450 induction methods and from untreated controls were prepared as described previously.[13] Microsomal P450 contents were determined spectrally by the established method.[14] Protein concentrations were estimated by using a bicinchoninic acid (BCA) protein assay kit (Pierce, Rockford, IL, U.S.A.). Pooled liver microsomes from humans (H150) were obtained from BD Biosciences (Woburn, MA, U.S.A.); the human subjects might not be exposed by 1,4-dioxane. Typical P450 substrates, their reac-

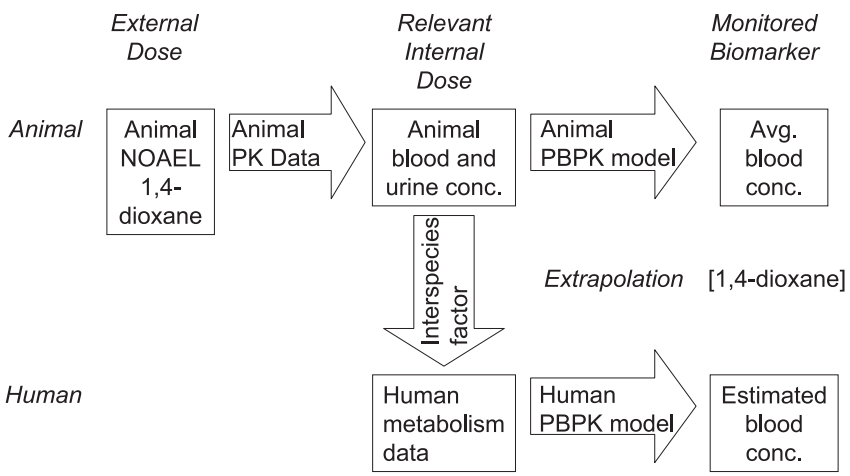

Fig. 2. Approach for Calculating Blood-based Biomonitoring Equivalents for 1,4-dioxane
PK, pharmacokinetics.

Table 1. Parameters Used for the rat PBPK Model

Parameter	Symbol	1,4-Dioxane	Unit
Octanol-water partition coefficient	$\log P$	−0.31	
Hepatic intrinsic clearance	$CL_{h,\mathrm{int}}$	0.0244	l/h
Liver-plasma concentration ratio	$K_{p,h}$	0.692	—
Renal clearance	CL_r	0.000290	l/h
Plasma unbound fraction	$f_{u,p}$	0.806	—
Ratio of the blood to plasma concentration	R_b	1.00	—
Volume of systemic circulation	V_1	0.0810	l
Hepatic volume	V_h	0.00850	l
Hepatic blood flow rate of systemic circulation to the tissue compartment	Q_h	0.853	l/h
Absorption rate constant	k_a	0.280	h^{-1}
Fraction absorbed × intestinal availability	F_aF_g	1.00	—
Dose	Dose	125	mg

tion products, and other reagents used in this study were obtained from sources described previously or were of the highest quality commercially available.[13, 15)]

Typical P450-dependent marker oxidation activities were measured in liver microsomes in rats to evaluate enzyme inductions on treatment with 1,4-dioxane. Activities for the *O*-dealkylation of ethoxyresorufin (20 μM, for CYP1A) and pentoxyresorufin (100 μM, CYP2B) and for testosterone 7α-hydroxylation (200 μM, CYP2A), tolbutamide methyl hydroxylation (1000 μM, CYP2C), bufuralol 1′-hydroxylation (20 μM, CYP2D), chlorzoxazone 6-hydroxylation (50 μM, CYP2E), and midazolam 1′- and 4-hydroxylation (100 μM, CYP3A) were assayed according to the described HPLC methods.[13, 16, 17)]

1,4-Dioxane Determinations in Biological Samples from Rats —— Plasma and urine samples from individual rats were diluted 10 times with water. 1,4-Dioxane concentrations in these samples were measured by a gas chromatograph/mass spectrometer (GC/MS) with a head-space sampler system as reported previously (GCMS-QP2010, Shimadzu, Kyoto, Japan)[8)] equipped with a CP-PORABOND Q column (25 m × 0.24 mm × 3 μm, Chrompack). To determine the concentrations, the intensities of the precursor ions for 1,4-dioxane (m/z = 88) and internal standard 1,4-dioxane-d_3 (m/z = 96) were used.

Human Metabolic Study —— Elimination rates of 1,4-dioxane in liver microsomes from humans and rats were measured by the GC/MS system described 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 (50 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 1,4-dioxane Concentrations by PBPK Modeling with Suitable Parameters —— A simplified PBPK model was set up as described previously.[8, 18)] 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,[19)] $K_{p,h}$ was estimated from these two values (Appendix A), and R_b was assumed to be 1.0 (blood and plasma concentrations are assumed to be equal). Parameter values which represent physiological properties such as hepatic volumes and blood flow rates in rats or humans were taken from the literature.[8, 18)]

Experimental plasma concentrations of 1,4-dioxane were analyzed by WinNonlin software (Professional version 5.01) with a one-compartment model and yielded primary k_a, elimination constant (k_{el}). Values of total clearance (CL_{tot}), hepatic clearance (CL_h), $CL_{h,\mathrm{int}}$, and V_1 were also calculated (Appendix B). Subsequently, final parameter values 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 shown in Fig. 3:

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

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

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

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

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.

To define a simplified PBPK model for 1,4-dioxane in humans based on the rat PBPK model, we used relevant liver microsomes and physiological parameters (CL_r, k_a, and V_1) derived from the literature and applied the systems approach to fit them into the traditional parallelogram for risk assessment,[2] as shown in Fig. 2 (Appendix C). The *in vivo* $CL_{h,\text{int}}$ of 1,4-dioxane in humans was estimated by multiplying the calculated initial parameter value for *in vitro* $CL_{h,\text{int}}$ in humans by the ratio of *in vivo* to *in vitro* $CL_{h,\text{int}}$ values in rats, as mentioned above for modeling in rats. Then, the final parameters for the PBPK model in humans were calculated using these initial values by the methods in Appendix C and are shown in Table 2. As was done for the rat model, systems of differential equations were solved to obtain concentrations in each model compartment in humans.

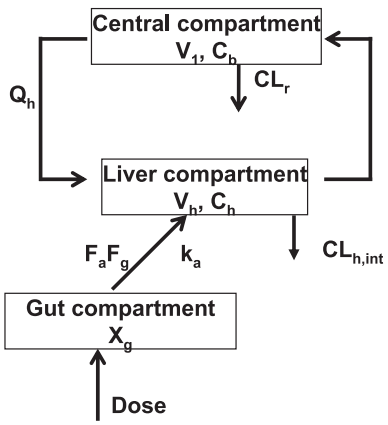

Fig. 3. PBPK Model Established in this Study for Rats and Humans

RESULTS

PBPK Model for Rats Orally Treated with 1,4-dioxane

To obtain detailed kinetic parameters, male rats were orally treated with 1,4-dioxane according to the protocol for general repeated exposure tests. Figure 3 shows the mean levels of 1,4-dioxane in blood and urine from rats after the final treatment of 14 daily repeated doses of 1,4-dioxane (500 mg/kg). 1,4-Dioxane was rapidly absorbed and immediately cleared within a day (Fig. 4A). Urinary excretion of 1,4-dioxane was almost complete within 24 hr after the final repeated administration (Fig. 4B). Renal clearance (CL_r) values of 1,4-dioxane were calculated from the amount excreted into the urine (2.25 mg) divided by the area under the blood curve

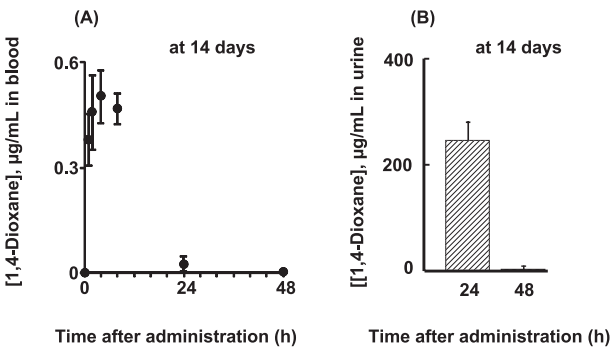

Fig. 4. PK Profiles in Rats *p.o.* Treated with 1,4-dioxane

1,4-Dioxane concentrations in blood (A) and urine (B) were determined in rats treated with 1,4-dioxane (500 mg/kg per day) after the final administration of 14 daily doses.

Table 2. Parameters Used for the Human PBPK Model

Parameter	Symbol	1,4-Dioxane	Unit
Hepatic intrinsic clearance	$CL_{h,\text{int}}$	1.76	l/h
Renal clearance	CL_r	0.0873	l/h
Volume of systemic circulation	V_1	23.7	l
Hepatic volume	V_h	1.50	l
Hepatic blood flow rate of systemic circulation to the tissue compartment	Q_h	96.6	l/h
Absorption rate constant	k_a	0.208	h^{-1}
Dose	Dose	35000	mg

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

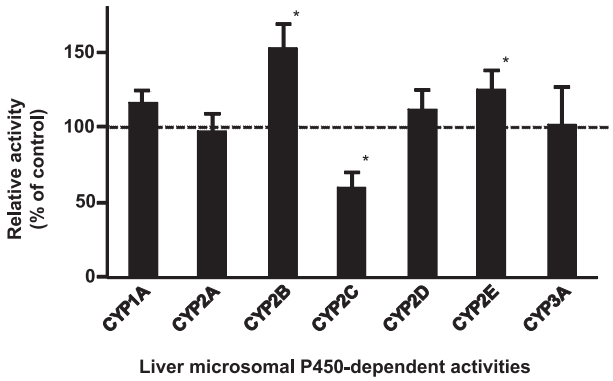

Fig. 5. Liver Microsomal P450-dependent Activities after 1,4-dioxane *i.p.* Treatment

Control activities were taken from liver microsomes from untreated rats. Data columns with bars show mean ± S.D. (n = 4). Significant differences compared with the control activities: $^{*}p < 0.05$. The *i.p.* treatment was conducted under the typical P450 induction by *i.p.* treatment with β-naphthoflavone, phenobarbital, and dexamethazone.[13)]

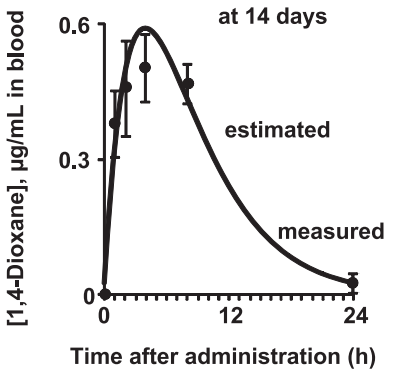

Fig. 6. Measured and Estimated 1,4-dioxane Blood Concentrations in Rats after Daily Oral Administration (500 mg/kg) for 14 days

Data points with bars represent experimental mean ± S.D. (n = 5) as shown in Fig. 4A. The curve shows concentrations estimated by PBPK modeling.

Table 3. *In Vitro* Intrinsic Clearance of 1,4-dioxane Determined Using Liver Microsomes

Enzyme source	Clearance, µl/min per mg protein	l/h[a)]
Rats, untreated[b)]	45 ± 13	0.805
Rats treated with 1,4-dioxane[b)]	20 ± 9	0.313
Pooled human livers	6.4	22.9

1,4-Dioxane (50 µM) was incubated with rat or human liver microsomes in the presence of an NADPH-generating system. The elimination rates of 1,4-dioxane were determined by GC/MS. *a*) Estimated clearance values were extrapolated using the following values: 40 mg liver microsomal protein per 1 g liver and 10 g liver weight per 0.25 kg of rat bw or 1.5 kg liver per 70 kg of human bw. *b*) Mean ± S.D. (n = 4) values using liver microsomes from individual rats *i.p.* pretreated with 1,4-dioxane (500 mg/kg) daily for 3 days or from untreated controls.

(7740 mg·h/l), giving 0.290 ml/h. Primary hepatic clearance values of 1,4-dioxane were obtained by subtracting CL_r from the total clearance. Values of the plasma unbound fraction ($f_{u,p}$) of 1,4-dioxane were calculated to be 0.806 by *in silico* estimation with SimCYP (Table 1).

P450 induction in rat liver microsomes was investigated after intraperitoneal treatment with 1,4-dioxane for 3 days (Fig. 5). Judging from typical P450-dependent drug oxidation activities, P450 induction or suppression by repeated treatments with 1,4-dioxane seemed to be within 50–150%, although CYP2B- and CYP2E-mediated activities were significantly induced and CYP2C-dependent activity was significantly decreased (Fig. 5). Consequently, final parameters such as hepatic intrinsic clearance ($CL_{h,\mathrm{int}}$), volume of systemic circulation (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 software to give 0.0244 l/h, 0.0810 l, and 0.280 h^{-1} and are shown in Table 1. By running the rat PBPK modeling system shown in Fig. 3, the blood concentration curves of 1,4-dioxane were estimated after repeated oral administration with 125 mg of 1,4-dioxane to a rat (250 g bw); the curve is shown in Fig. 6. The estimated *in silico* concentration curve of 1,4-dioxane is shown with the *in vivo* experimental data points.Minimal accumulation was found by the present rat PBPK model 24 hr after daily treatment with 1,4-dioxane in rats.

Human PBPK Model Supported by *In Vitro* Hepatic Clearance Experiments

Hepatic clearance of 1,4-dioxane *in vitro* was determined in pooled human liver microsomes and compared with data from liver microsomes from rats pretreated with 1,4-dioxane and from untreated controls (Table 3). Hepatic clearance of 1,4-dioxane in human liver microsomes was calculated to be 6.4 µl/min per mg protein; this was apparently lower than the values for rat livers. Subsequently, the hepatic intrinsic clearance of 1,4-dioxane in humans was found be 22.9 l/h in an *in vitro* study using the biological coefficients already established. The intrinsic clearance values of 1,4-dioxane based on rat *in vivo* (Table 1) and rat *in vitro* (Table 3) experiments were different; this ratio (0.0244/0.313) was used as the compensating factor for estimating *in vivo* hepatic intrinsic clearance in humans. Finally,

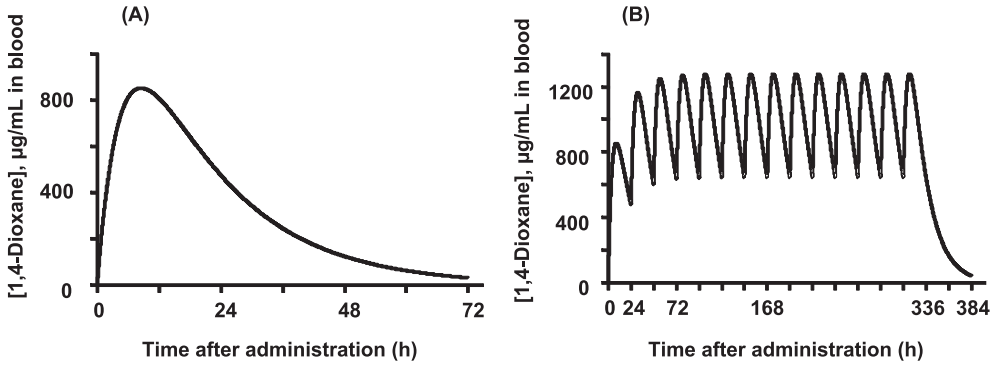

Fig. 7. 1,4-Dioxane Concentrations Hypothetically Modeled in Humans after Single (A) or Multiple (B) Oral Administrations (500 mg/kg per day) Estimated Using the PBPK Model
Little accumulation was observed for multiple doses.

a value of 1.76 l/h for the 1,4-dioxane 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 the estimated human blood concentrations after modeling single and repeated oral administration of 1,4-dioxane (500 mg/kg). The apparent maximum concentrations of 1,4-dioxane were estimated to be approximately 800 μg/ml after a single treatment (Fig. 7A). When daily administration of 1,4-dioxane for 14 days was modeled, accumulation was found by the present human PBPK model (Fig. 7B).

DISCUSSION

It is generally accepted that PBPK modeling could be of use for understanding the relationship between chemical exposure and concentrations in body fluids (Fig. 2). 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 1,4-dioxane in humans (Fig. 3); the model was based on physiological parameters derived from the literature, coefficients derived *in silico*, metabolic parameters determined *in vitro* using relevant liver microsomes, and *in vivo* experiment-supported PBPK modeling in rats. 1,4-Dioxane metabolic clearance is believed to be generally dependent on P450s, but there is no information regarding P450 isoforms in the 1,4-dioxane metabolism in rats or humans and its P450 induction/inhibition so far.[7,8] P450 induction or suppression by repeated treatments with 1,4-dioxane seemed to be limited in the present study, in spite of some increased or deceased marker oxidation activities (Fig. 5). Judging from effects of 1,4-dioxane on its some suppressed metabolic clearance in rat liver microsomes (Table 3), metabolic clearance of 1,4-dioxane may be mediated partly by CYP2C enzymes in male rats. The developed PBPK model for 1,4-dioxane in rats simply consisted of three compartments, including the gut as the chemical absorption compartment, the liver as the metabolizing compartment, and the general circulation as the central compartment for 1,4-dioxane (Fig. 3). 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. 4) and *in vitro* clearance experiments with rat and human liver microsomes (Table 3) in the present study.

A critical review of the potential carcinogenicity of the weak genotoxicant 1,4-dioxane has indicated that the relevance of nasal cavity tumors to human exposure via the oral route is questionable.[9] A six-compartment PBPK model for 1,4-dioxane in humans has been reported.[8] Although oral administration of chemicals is a key route of exposure based on toxicology testing,[10] chemical concentrations absorbed from other routes, such as inhalation, could be handled in a similar PBPK modeling system. There are limited human data that can be used to validate the human model. Sweeney *et al.*[8] have reported a prediction of human subjects data for 50 ppm inhalation for 6 hr exposure to 1,4-dioxane. In that report,[8] plasma levels of 1,4-dioxane modeled in humans show some accumulation tendency

similar to our predicted values after a single administration of 1,4-dioxane, as shown in Fig. 7A. In this context, some validation of the human PBPK model is needed in future study, based on the good agreement of measured and estimated blood concentrations in the rat PBPK model in this study.

Human biomonitoring is important for many aspects of environmental health.[3, 20] Evaluation of a previously developed rat model was performed by comparing the blood concentrations predicted by PBPK modeling *in silico* and experimental biokinetic values from plasma and urine obtained from rats *in vivo* after repeated oral treatment with 1,4-dioxane at an NOAEL. To overcome the species differences in animals and humans, the traditional parallelogram technique used in systems biology,[21] namely the concept of animal-human-*in vitro*-*in vivo* parallelogram in the risk assessment system[22, 23], 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 1,4-dioxane is useful for a forward dosimetry approach in rats and humans to estimate blood concentrations of 1,4-dioxane and other related 1,4-dioxane-like 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):[24]

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

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

$$P = 10^{\log P} \tag{A2}$$

$f_{u,h}$ 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:

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

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 following equations.

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

$$CL_h = \frac{Dose \times Q_h - AUC \times CL_r \times Q_h}{AUC \times Q_h + Dose} \tag{B1}$$

$$\frac{CL_{\mathrm{tot}}}{F} = \frac{CL_h + CL_r}{1 - \frac{CL_h}{Q_h}} = \frac{Dose}{AUC} \tag{B2}$$

where AUC is the area under the curve.

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

$$F = F_a \times F_g \times F_h \tag{B3}$$

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 $F_a F_g = 1.0$).

$$F' = F_a F_g \times F_h = F_h = 1 - \frac{CL_h}{Q_h} \tag{B4}$$

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):

$$V'_1 = (V_d/F) \times F' \tag{B5}$$

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):

$$CL'_{h,\mathrm{int}} = \frac{R_b}{f_{u,p}} \times \frac{Q_h \times CL_h}{Q_h - CL_h} \tag{B6}$$

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

$$CL_h = CL_{\mathrm{tot}} - CL_r \tag{B8}$$

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):

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

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:

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

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

The human systemic circulation volume ($V_{1,human}$) was estimated from Eqs. (C3) and (C4), where $V_{h,rat}$, $V_{h,human}$, $V_{b,rat}$, and $V_{b,human}$ were 0.0085 l, 1.5 l, 0.016 l, 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).[25)]

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

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