Letter

Plasma, liver, and kidney exposures in rats after oral doses of industrial chemicals predicted using physiologically based pharmacokinetic models: A case study of perfluorooctane sulfonic acid

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ABSTRACT — A simplified physiologically based pharmacokinetic (PBPK) model consisting of chemical receptor, metabolizing and/or excreting, and central compartments was recently proposed. In the current study, this type of PBPK model was set up for perfluorooctane sulfonate, an environmental toxicant with liver effects, as a model compound; the model was then used to estimate tissue concentrations. The pharmacokinetic parameter input values for the model were calculated to give the best fit to reported/measured blood substrate concentrations in rats. The maximum concentrations and areas under the concentration versus time curves in plasma, liver, and kidney extrapolated using PBPK models for perfluorobutane sulfonic acid, perfluorohexane sulfonic acid, and perfluorooctane sulfonic acid were consistent with the reported mean values in rats. Using the rat models and scaled-up human PBPK models, some accumulation of perfluorooctane sulfonic acid in plasma and liver was seen after repeated doses. The reported 50th and 95th percentile concentrations of perfluorooctane sulfonic acid in human blood (0.0048 and 0.0183 ng/mL, respectively) in the general population underwent reverse dosimetry analysis using our PBPK models. These human blood concentrations potentially imply exposures of 0.041 and 0.16 $\mu\text{g/kg/day}$, respectively, for 90 days, values that are roughly similar to the reference dose (0.02 $\mu\text{g/kg/day}$) with an uncertainty factor of 30. These results indicate the relatively good estimates for tissue and blood exposures of chemical substrates after oral doses generated using the latest PBPK models.

Key words: AUC, C_{max} , PBPK modeling, PFOS, Reference dose level

INTRODUCTION

In vivo toxicity studies are extensively characterized by a substance's no-observed-effect level (NOEL) and/or the lowest-observed-effect level (LOEL) for a given toxic outcome (Martin *et al.*, 2009). To investigate the external chemical doses that produce *in vivo* toxicity, an empirical association between the internal concentrations of chemicals and the external doses could be explored (Wambaugh *et al.*, 2013). Physiologically based pharmacokinetic (PBPK) modeling is generally accepted as a method to estimate toxicokinetics (Wetmore *et al.*, 2012; Wambaugh *et al.*, 2015). In addition to full PBPK models, simplified PBPK models have been established that consist of

a chemical receptor compartment, metabolizing and/or excreting compartments, and a central compartment (Kamiya *et al.*, 2019, 2020). However, there are limited numbers of industrial chemicals for which sufficient *in vivo* toxicokinetic data have been reported to allow PBPK modeling or risk assessment of potential hazards (Bell *et al.*, 2018; Sayre *et al.*, 2020). PBPK forward dosimetry can provide the plasma/tissue concentrations of drugs and chemicals after oral dosing, thereby facilitating estimates of toxic potential as a part of risk assessment. Moreover, using this approach, the internal concentrations *in vivo* that cause chemical bioactivities marking the transition from external NOEL or LOEL doses could be estimated.

Perfluorinated compounds with long-chain fatty

acid analogs in which fluorine atoms take the place of all hydrogen atoms are well known chemical toxicants (Lau *et al.*, 2004; Harris and Barton, 2008; Benskin *et al.*, 2009a). These compounds are of particular interest because measurable blood levels are detected in the general population worldwide (Lau *et al.*, 2007; Wambaugh *et al.*, 2013). Because extensive exposure to perfluoroalkyl substances has been observed in many countries (Silva *et al.*, 2020), a multicompartiment pharmacokinetic model was set up for perfluorinated compounds to facilitate integrated probabilistic risk assessment (Wambaugh *et al.*, 2013). In this study, we investigated tissue concentrations of perfluorooctane sulfonic acid as a model perfluoroalkyl compound (Fig. 1) to validate our latest simplified PBPK models (Kamiya *et al.*, 2020). We report

herein consistent results between the reported/measured values and the estimated values generated by PBPK modeling in terms of the plasma and hepatic/renal concentrations after virtual oral doses of perfluorooctane sulfonic acid and its related compounds.

MATERIALS AND METHODS

The details for establishing PBPK models were recently reported (Kamiya *et al.*, 2020). The values used for the hepatic/renal blood flow rates (Q_h , Q_r) in rats were 0.853 L/hr, and the hepatic and renal volumes, respectively, were 8.5 mL and 3.7 mL, according to the literature (Brown *et al.*, 1997; Davies and Morris, 1993). The octanol–water partition coefficients ($\log P$) and plasma

Fig. 1. Previously reported (plotted points) and currently estimated plasma, hepatic, and renal concentrations (lines) of perfluorobutane sulfonic acid, perfluorohexane sulfonic acid, and perfluorooctane sulfonic acid using PBPK modeling after oral doses. Plasma (solid lines), hepatic (broken lines), and renal (dashed lines) concentrations of the three chemicals estimated using PBPK models with the parameters given in Table 1 are shown after virtual oral doses of 1.0 mg/kg (A–C). Reported plasma (circles), hepatic (triangles), and renal (squares) concentrations normalized by dose (1.0 mg/kg) in rats were taken from the data set for perfluorobutane sulfonic acid (20 mg/kg, A), perfluorohexane sulfonic acid (16 mg/kg, B), and perfluorooctane sulfonic acid (20 mg/kg, C) (Sayre *et al.*, 2020). Plasma (gray lines) and hepatic (black lines) concentrations of perfluorooctane sulfonic acid are shown after multiple virtual oral doses (0.02 µg/kg/day, the reference dose) virtually administered to rats (D) and humans (E) for 90 days.

unbound fractions ($f_{u,p}$) of the test compounds were estimated using *in silico* program tool; the blood-to-plasma concentration ratios (R_b) and liver (or kidney)-to-plasma concentration ratios ($K_{p,h}$ or $K_{p,r}$) were calculated from their $f_{u,p}$ and $\log P$ values (Takano *et al.*, 2010; Uchimura *et al.*, 2008; Kamiya *et al.*, 2020). The input parameters for our simplified PBPK models, i.e., the absorption rate constant (k_a), the volume of the systemic circulation (V_1), and the hepatic intrinsic clearance ($CL_{h,int}$), for perfluorooctane sulfonic acid and related compounds were computed in a similar way to that described in our recent study (Kamiya *et al.*, 2020) to give the best fit to measured blood substrate concentration values obtained by a US research group (Sayre *et al.*, 2020). The fraction absorbed $\times$ intestinal availability ($F_a \cdot F_g$), hepatic clearance (CL_h), and renal clearance (CL_r) for individual compounds were similarly calculated (Kamiya *et al.*, 2020) and are shown in Table 1. A system of differential equations was solved to determine the *in vivo* concentrations:

$$\begin{aligned} \frac{dX_g}{dt} &= -k_a \cdot X_g \text{ when at } t = 0, X_g(0) = F_a \cdot F_g \cdot \text{dose} \\ V_h \frac{dC_h}{dt} &= k_a \cdot X_g - \frac{Q_h \cdot C_h \cdot R_b}{K_{p,h}} - CL_{h,int} \cdot \frac{C_h}{K_{p,h}} \cdot f_{u,p} + Q_h \cdot C_b \\ V_1 \frac{dC_b}{dt} &= -(Q_h + Q_r) \cdot C_b + \frac{Q_h \cdot C_h \cdot R_b}{K_{p,h}} + \frac{Q_r \cdot C_r \cdot R_b}{K_{p,r}} \\ V_r \frac{dC_r}{dt} &= Q_r \cdot C_b - \frac{Q_r \cdot C_r \cdot R_b}{K_{p,r}} - CL_r \cdot \frac{C_r}{K_{p,r}} \cdot f_{u,p} \end{aligned}$$

where X_g , V_h , V_r , C_h , C_r , and C_b are the amount of compound in the gut; the liver and kidney volumes; and the hepatic, renal, and blood substrate concentrations, respectively. The maximum concentrations (C_{max} , ng/mL or ng/g) and the areas under the concentration versus time curves from the moment of virtual oral dosing (AUC, ng·hr/mL or ng·hr/g) in plasma, liver, and kidney were also calculated after virtual single or multiple oral administrations of normalized 1.0-mg/kg doses using PBPK modeling (Kamiya *et al.*, 2020).

To establish a simplified human PBPK model based on the rat PBPK model, the rodent k_a value was multiplied by 0.744 to give the human k_a value (Takano *et al.*, 2010; Kamiya *et al.*, 2020). The human systemic circulation volume, CL_h , and CL_r was estimated as described previously (Takano *et al.*, 2010; Kamiya *et al.*, 2020), where $BW_{\text{rodent}} = 0.25$ kg (rat) and $BW_{\text{human}} = 70$ kg:

$$CL_{h \text{ or } r, \text{human}} = \frac{CL_{h \text{ or } r, \text{rodent}}}{\text{Body weight}_{\text{rodent}}^{\left(\frac{2}{3}\right)}} \cdot \text{Body weight}_{\text{human}}^{\left(\frac{2}{3}\right)}$$

RESULTS AND DISCUSSION

Perfluorooctane sulfonic acid and the two related compounds shown in Fig. 1 have reported blood, hepatic, and renal concentrations measured after oral administrations in rats (Sayre *et al.*, 2020). These reported data and the PBPK-modeled plasma, hepatic, and renal concentration curves of these compounds are shown in Fig. 1; the PBPK models used the input pharmacokinetic parameters determined in this study (Table 1). Because the pharmacokinetic input parameter values of chemicals for animal PBPK models were calculated as described previously to give the best fit to measured blood substrate concentrations (Kamiya *et al.*, 2019, 2020), the virtual output C_{max} and $AUC_{0-24 \text{ hr or } 0-12 \text{ hr}}$ values in plasma extrapolated using the PBPK models for perfluorobutane sulfonic acid, perfluorohexane sulfonic acid, and perfluorooctane sulfonic acid were consistent with the reported mean values in rats. These observations confirmed the relatively good fit of the results (within a two-fold range of observed values) as shown in Table 1.

The rates of apparent clearance of perfluorobutane sulfonic acid (Fig. 1A), perfluorohexane sulfonic acid (Fig. 1B), and perfluorooctane sulfonic acid (Fig. 1C) from plasma in rats were inversely related to the order of their lyophilic properties as shown in Table 1. The hepatic and renal concentrations of perfluorobutane sulfonic acid (Fig. 1A) and perfluorohexane sulfonic acid (Fig. 1B) were lower than the plasma concentrations of those chemicals, as previously reported and currently modeled. In contrast, tissue concentrations of perfluorooctane sulfonic acid (Fig. 1C) were higher than the plasma concentrations, as previously reported and currently modeled (Table 1).

Under the present conditions, the virtual hepatic C_{max} and $AUC_{0-24 \text{ hr}}$ or $AUC_{0-12 \text{ hr}}$ modeled values for perfluorobutane sulfonic acid and perfluorooctane sulfonic acid were within two-fold ranges of the measured values, whereas those for perfluorohexane sulfonic acid were within a 2.4-fold range, indicating the relatively good fit of the results for hepatic exposures as shown in Fig. 1. Virtual renal C_{max} or $AUC_{0-24 \text{ hr}}$ or $AUC_{0-12 \text{ hr}}$ output values for perfluorobutane sulfonic acid and perfluorooctane sulfonic acid were also within two-fold ranges of the empirically obtained values, whereas those for perfluorohexane sulfonic acid were within a four-fold range (Table 1). For human PBPK modeling, the human k_a , V_1 , *in vivo* $CL_{h,int}$, CL_h , and CL_r values for perfluorooctane sulfonic acid were 0.173 1/hr, 12.5 L, 0.450 L/hr, 0.0243 L/hr, and 0.00245 L/hr, respectively. The reported long human half-lives of years are in contrast to the half-lives of days for

Table 1. Chemical properties, pharmacokinetic input parameters for PBPK models, and forward dosimetry model output for rats after virtual oral administration of 1.0 mg/kg.

Pharmacokinetic parameters	Perfluorobutane sulfonic acid	Perfluorohexane sulfonic acid	Perfluorooctane sulfonic acid
Model input parameters			
Molecular weight	300	400	500
Octanol–water partition coefficient ($\log P$)	1.34	1.81	2.28
Plasma unbound fraction	0.111	0.078	0.054
Blood–plasma concentration ratio	0.841	0.81	0.78
Liver–plasma concentration ratio	0.648	0.972	1.56
Fraction absorbed $\times$ intestinal availability	1	1	1
Absorption rate constant, 1/hr	2.62 ± 0.24	0.669 ± 0.056	0.233 ± 0.070
Volume of systemic circulation, L	0.0848 ± 0.0029	0.0169 ± 0.0009	0.0252 ± 0.0068
Hepatic intrinsic clearance, L/hr	0.147 ± 0.004	0.00859 ± 0.00078	0.0105 ± 0.0037
Hepatic clearance, L/hr	0.016	0.00067	0.000569
Renal clearance, L/hr	0.00162	0.0000675	0.0000572
Reported/observed levels ^a			
$C_{\max}$ in plasma, $\mu\text{g/mL}$	2.50	8.30	5.46
AUC in plasma, $\mu\text{g}\cdot\text{hr/mL}$	15.2	163	110
$C_{\max}$ in liver, $\mu\text{g/g}$	3.21	3.76	7.74
AUC in liver, $\mu\text{g}\cdot\text{hr/g}$	18.8^b	74.5	162
$C_{\max}$ in kidney, $\mu\text{g/g}$	1.07	2.35	5.16
AUC in kidney, $\mu\text{g}\cdot\text{hr/g}$	6.21^b	45.5	107
Estimated values			
$C_{\max}$ in plasma, $\mu\text{g/mL}$	$2.48 (0.99)^c$	$8.11 (0.98)$	$4.69 (0.86)$
AUC in plasma, $\mu\text{g}\cdot\text{hr/mL}$	$15.0 (0.99)$	$158 (0.97)$	$96.1 (0.88)$
$C_{\max}$ in liver, $\mu\text{g/g}$	$1.70 (0.53)$	$8.99 (2.4)$	$9.77 (1.3)$
AUC in liver, $\mu\text{g}\cdot\text{hr/g}$	$9.35^b (0.50)$	$176 (2.4)$	$200 (1.2)$
$C_{\max}$ in kidney, $\mu\text{g/g}$	$1.70 (1.6)$	$8.99 (3.8)$	$9.77 (1.9)$
AUC in kidney, $\mu\text{g}\cdot\text{hr/g}$	$9.35^b (1.5)$	$175 (3.9)$	$200 (1.9)$

^a Reported plasma and tissue concentrations normalized by dose (1.0 mg/kg) were taken from the data set published by Sayre *et al.*, 2020.

^b $\text{AUC}_{0-12\text{ hr}}$ instead of $\text{AUC}_{0-24\text{ hr}}$ values.

^c Values in parentheses are ratios to the reported values.

male rats (Lau *et al.*, 2007). Forward and reverse dosimetry were carried out in this study under the assumption of similar *in vitro* $CL_{h,int}$ values (Benskin *et al.*, 2009b) for rats and human PBPK models. Using the newly established PBPK models for rats and humans, some accumulation of perfluorooctane sulfonic acid in plasma and liver was indicated after repeated doses (Fig. 1D and 1E).

Perfluorooctane sulfonic acid is a relatively well-studied chemical with LOELs of ~ 1 mg/kg in adult rodents for liver effects and for developmental endpoints in offspring (Wambaugh *et al.*, 2013). The levels of the repeated virtual doses of perfluorooctane sulfonic acid (0.041 and 0.16 $\mu\text{g/kg/day}$) for 90 days in humans were calculated via reverse dosimetry using the 50th and 95th percentiles of human blood levels from 2015–2016 (0.0048 and 0.0183 ng/mL), respectively, published in a biomonitoring report from the USA (Fourth National Report on Human Exposure to Environmental Chemicals Updated Tables, January 2019, <https://www.cdc.gov/exposurereport/>). These 50th and 95th percentile concentrations in the blood potentially imply exposures roughly similar to the reference dose (0.02 $\mu\text{g/kg/day}$) with an uncertainty factor of 30 recommended by regulatory authorities (Silva *et al.*, 2020). In-depth forward and reverse assessment of perfluorooctane sulfonic acid levels could be facilitated by adopting the current human PBPK model.

In conclusion, in this study, by using our latest simplified PBPK modeling system, we confirmed good predictions of tissue concentrations (Kamiya *et al.*, 2020) compared with the measured plasma, hepatic, and renal concentrations of perfluorooctane sulfonic acid and two related compounds. Chemical exposure levels in plas-

PBPK modeling of PFOS in humans

ma, liver, and kidney after oral doses could be estimated via pharmacokinetic modeling. After further investigations and validation processes, this approach should prove to be a useful addition to computational toxicology. This type of PBPK model could estimate and help to evaluate the potential risk from multiple exposures of industrial chemicals in chemical toxicology.

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Conflict of interest---- The authors declare that there is no conflict of interest.

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