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Inhalation Dosimetry Modeling with Decamethylcyclopentasiloxane in Rats and Humans

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Decamethylcyclopentasiloxane (D_5), a volatile cyclic methyl siloxane (VCMS), is used in industrial and consumer products. Inhalation pharmacokinetics of another VCMS, octamethylcyclotetrasiloxane (D_4), have been extensively investigated and successfully modeled with a multispecies physiologically based pharmacokinetic (PBPK) model. Here, we develop an inhalation PBPK description for D_5, using the D_4 model structure as a starting point, with the objective of understanding factors that regulate free blood and tissue concentrations of this highly lipophilic vapor after inhalation in rats and humans. Compared with D_4, the more lipophilic D_5 required deep compartments in lung, liver, and plasma to account for slow release from tissues after cessation of exposures. Simulations of the kinetics of a stable D_5 metabolite, HO-D_5, required diffusion-limited uptake in fat, a deep tissue store in lung, and its elimination by fecal excretion and metabolism to linear silanols. The combined D_5/HO-D_5 model described blood and tissue concentrations of parent D_5 and elimination of total radioactivity in single and repeat exposures in male and female rats at 7 and 160 ppm. In humans, D_5 kinetic data are more sparse and the model structure though much simplified, still required free and bound blood D_5 to simulate exhaled air and blood time courses from 1 h inhalation exposures at 10 ppm in five human volunteers. This multispecies PBPK model for D_5 highlights complications in interpreting kinetic studies where chemical in blood and tissues represents various pools with only a portion free. The ability to simulate free concentrations is essential for dosimetry based risk assessments for these VCMS.

Key Words: **decamethylcyclopentasiloxane; D_5; inhalation pharmacokinetics; PBPK modeling; dose metrics; lipophilic volatiles; free concentrations in blood.**

Decamethylcyclopentasiloxane (D_5), a volatile cyclic methyl siloxane (VCMS), is used in a broad range of consumer and industrial products including some personal care products. The general population may be exposed to low levels of D_5 by skin contact and inhalation through use of these products. In the workplace exposures occur by inhalation during the production of D_5 and by contact with products containing this compound. A related compound, octamethylcyclotetrasiloxane (D_4), has been widely used in the past, but its usage is diminishing. High concentration exposures to D_4 caused several biological responses in rats, including hepatic hypertrophy (McKim *et al*., 2001a), effects on reproduction via diminishing luteinizing hormone release (Siddiqui *et al*., 2007a), and estrogenicity (McKim *et al*., 2001b). Exposures of rats to D_5 have not demonstrated these reproductive or estrogenic responses (Quinn *et al*., 2007; Siddiqui *et al*., 2007b), but did cause liver hypertrophy (McKim *et al*., 1999).

These VCMS are highly lipophilic with fat:blood partition coefficients (PCs) in excess of 500, leading to pharmacokinetic behaviors and tissue persistence after cessation of exposures (Andersen *et al*., 2001; Reddy *et al*., 2007) that are more complex than noted with volatile organic compounds (VOCs), such as styrene (Ramsey and Andersen, 1984) or vinyl chloride (Reitz *et al*., 1996). Even the pharmacokinetic behavior of a low molecular weight linear siloxane, hexamethyldisiloxane (HMDS), was heavily influenced by high lipophilicity: the fat:blood PC for HMDS is near 300 (Dobrev *et al*., 2008). In rats, hepatic responses to D_4, including enzyme induction and liver hypertrophy, were found to be more closely related to time course concentrations of free parent compound in the target tissue rather than to total liver D_4 (Sarangapani *et al*., 2002). This observation indicates that the relevant VCMS dose metric will be blood or tissue concentrations of *free* parent compounds. Due to analytical challenges with these materials, methodology does not exist that easily allows measurement of free concentration in blood or tissues separate from the bound pools.

For VCMS, physiologically based pharmacokinetic (PBPK) models will be necessary to interpret kinetic studies and

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distinguish free concentrations from total concentrations that also represent bound pools in blood and deep tissue stores. PBPK models have been applied to D_4 (Andersen *et al*., 2001) and HMDS (Dobrev *et al*., 2008) and a PBPK model for dermal application of D_5 in humans has been previously described (Reddy *et al*., 2007). Recently, plasma and tissue kinetics of inhaled D_5 have been reported in rats following single and multiple exposures (Tobin *et al*., 2008). Plasma and exhaled breath data are also available from a human volunteer study with inhaled D_5 that was conducted at the University of Rochester. Our goal was to develop a PBPK model for inhaled D_5 in rats and humans that could estimate free blood and tissue concentrations of this compound for various exposure conditions. Such a model will be useful for risk assessment applications in assessing tissue responses based on measures of free, bioactive concentrations of this lipophilic compound.

MATERIALS AND METHODS

Rat experimental data. The PBPK model was developed using data for the disposition of radiolabeled and parent D_5 in male and female Fischer 344 rats following single and multiple exposures at 7 and 160 ppm (Tobin *et al*., 2008). A brief synopsis of the study design is included in Supplemental Materials (S-1).

Human experimental data. The data used to extend the rat PBPK model in humans were collected at the University of Rochester following protocol approval by the Human Subject Review Committee. The human use protocol, similar to that used with D_4 (Reddy *et al*., 2003; Utell *et al*., 1998), was approved in October 1994 and the experiments were completed between February and April 1996. There were five subjects: three men and two women. The women volunteers were required to have a negative pregnancy test before participation. A synopsis of this study, including a more detailed overview of the protocol consent form and study rationale is provided in Supplemental Materials (S-2).

Partition coefficients. PCs for D_5 (Table 1) were measured in various media using following methods used previously for D_4 and HMDS (Andersen *et al*., 2001; Dobrev *et al*., 2003). Fisher 344 rats (300 and 350 g) were anesthetized with isoflurane, blood was collected in heparinized tubes and samples of liver, kidney, brain, muscle from the hind legs, and perirenal fat were removed. Fat and other tissues were homogenized in 0.9% (wt/vol) NaCl (tissue/saline ratio of 1:1). A series of scintillation vials was placed in a large mouth canister with a screw top seal. The vials contained tissue homogenates, rat blood, corn/olive oil, and pure D_5 and water was placed the bottom of the larger container to maintain humidity. The canister was sealed and placed into a recirculated water bath at 37°C. Up to 48 h was allowed for complete air-phase and tissue equilibration with air sampling at 24 and 48 h through a port on the top of the canister using a 250-μl Hamilton gas-tight syringe. After air sampling, the canister was carefully opened and an aliquot of each sample (except the pure D_5) ranging from 0.8 to 1.5 g was transferred to a conical separatory glass tube (15 ml capacity) containing 5 ml reagent-grade tetrahydrofuran (THF). Extraction followed methods used with D_4 (Varaprath *et al*., 2000). The tubes were tightly capped and vortex-mixed at high-speed settings for 3 min. The samples were then centrifuged for 5 min and left overnight in a freezer. On the next day, the clear top layer of THF was carefully removed and transferred into a clean separatory glass tube. The extraction procedure was repeated by adding 3 ml of fresh solvent to the sample, and both THF extracts were combined. After a volume adjustment, D_5 was analyzed using an HP 6890 gas chromatograph (GC) equipped with a capillary column (HP-5, 30 m/0.32 mm with 0.25-μm film thickness) and flame ionization detector (FID). FID gases were hydrogen and synthetic air. Nitrogen, at a flow rate of 1.5 ml/min, was the carrier gas. The GC oven was held at initial temperature of 40°C for 6 min and heated at 10°C/min to a final temperature of 180°C. The injector port and detector temperature were 150°C and 250°C, respectively. One-microliter aliquots were injected in splitless mode. Data analysis was performed using Hewlett-Packard Chemstation software. The linearity of the detector response in the concentration range of interest was tested by developing a calibration curve for D_5 diluted in THF.

Model structure—rat. Although initial efforts were made to describe the D_5 data with the D_4 PBPK model (Andersen *et al*., 2001; Reddy *et al*., 2003), tissue and plasma elimination time courses were not adequately captured with the unchanged D_4 model. The discrepancies between D_4 model predictions and D_5 data were suggestive of more prolonged elimination from tissue depots. To capture these differences between the two VCMS it was necessary to include several other tissue subcompartments in with D_5. In addition, the presence of a stable, cyclic metabolite, referred to as HO-D_5, required connection between rates of oxidation of D_5 and production of the stable metabolite. In the final D_5 model structure (Fig. 1), the blood had one deep tissue compartment and the lung and liver had two deep tissue compartments, with D_5 moving between the tissue compartments and deep compartments by diffusional transport. There were also two fat compartments with diffusion-limited uptake. The slowly perfused compartment also had diffusion-limited uptake. As in the D_4 model structure, there was a pool of bound D_5 that was produced in the liver, transported in blood, and cleared from blood into the fat tissue in a manner representative of lipoprotein transport. This pool of blood VCMS is not available for exhalation, equilibration with tissue, or metabolism in the liver while being transported in blood (see submodel; bottom left, Fig. 1). Metabolism of D_5 to the HO-D_5 was modeled as a first-order process, occurring in the liver. With D_4, limited induction of metabolism was included at the highest exposure concentration (700 ppm). However, the tissue concentrations produced by 160 ppm D_5 were an order-of-magnitude lower than for D_4 and these concentrations were regarded as unlikely to increase metabolism.

The development of the PBPK model for D_5 followed the approaches used for a variety of volatiles in developing a model structure based on a blood:air PC with all chemical in blood available for transfer into other tissues during circulation through the organs. The laboratory studies determined plasma concentrations of D_5. If the plasma and whole blood concentrations were vastly different, this approach would be inappropriate. With D_5, the inhalation kinetic studies (Tobin *et al*., 2008) show that the plasma concentrations are about 80% of the whole blood concentrations of D_5. Thus, our model structure in which the calculations for whole blood are compared with plasma concentrations has a small, consistent error. However, the systematic error is small. As modeling with these compounds continues, there will be value in determining the PCs in both blood and plasma and separate calculations for both compartments.

The metabolite submodel for the stable HO-D_5 compound (right side; Fig. 1) also included a deep compartment in the lung and diffusion-limited uptake in fat. HO-D_5 clearance occurred by elimination into feces and by further metabolism in the liver. The subsequent metabolism of the VCMS produces a range of downstream biotransformation products, that is, short chain linear silanols (Varaprath *et al*., 1999, 2003). As done with these more water soluble linear silanols in the PBPK modeling for D_4, this mixed pool of silanols was described as a single chemical entity distributed to a central compartment and excreted into the urine. The equations for the D_5 rat inhalation model are provided in Supplemental Materials (S-3). Computer code for the rat and human models can be obtained from the corresponding author.

Model structure—human. Data in the human volunteers are sparse and provide information on plasma and exhaled breath time courses. The PBPK model for D_5 inhalation in humans was a simplified version of the rat model (Supplemental Materials, S-4). As with inhalation of D_4 in human volunteers, the model structure required a pool of unavailable D_5 in the blood that was produced in the liver, transported from liver into blood, and cleared from blood into fat tissue. Both the lung and liver had a deep compartment described with diffusional transport from the main tissue compartment. Metabolism of D_5 in

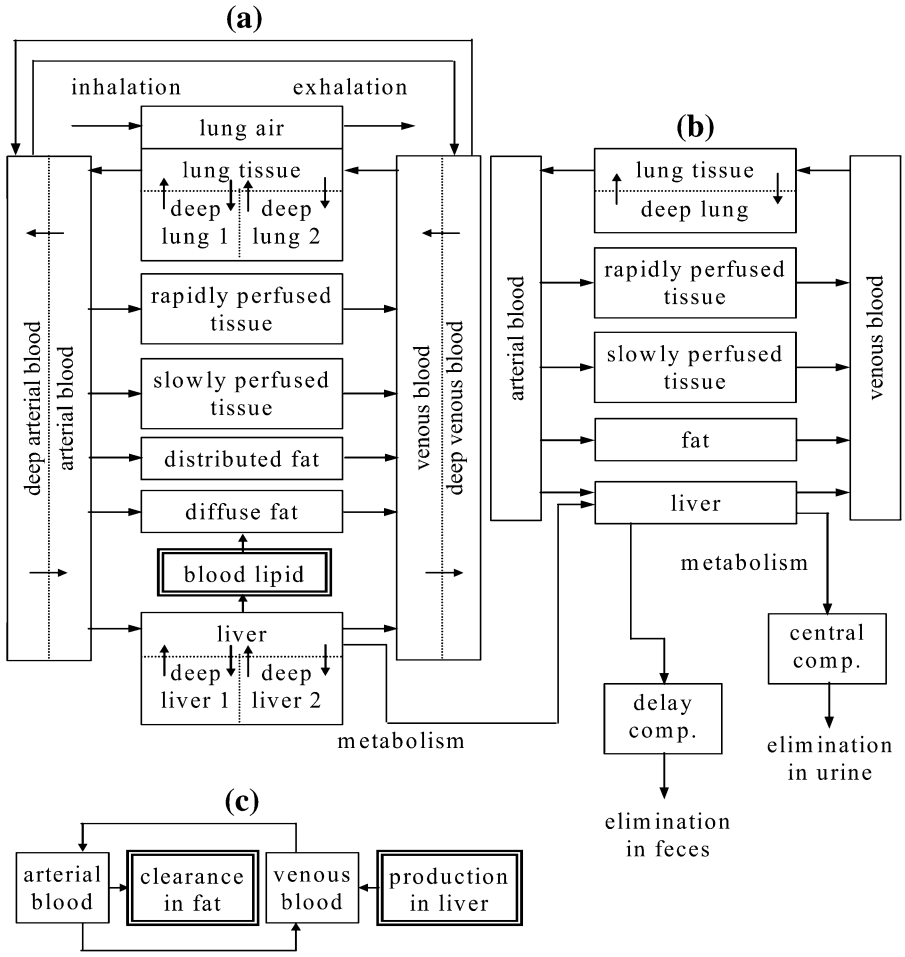

FIG. 1. Schematic diagram of (a) the D_5 PBPK model in rat, (b) the metabolite submodel, and (c) the submodel for the transport of D_5 in the mobile lipid pool. The double line designates a variable from a component in another section of the model.

humans was modeled as first-order hepatic clearance, and D_5 was also eliminated by exhalation. No data were available in humans to develop submodels for the linear silanols with D_5, as done previously with D_4 (Reddy *et al*., 2003).

Parameterization of the rat model. The PBPK model has physiological parameters (Table 2) and additional parameters estimated from experimental data (Table 3). Rat body weights were treated as a constant in the 15-day studies. An overview of the model fitting process is provided in a flowchart in Supplemental Materials (Supplemental Materials, **S-5 and S-6**). The general approach followed closely the processes described in fitting the D_4-inhalation model to various data sets (Andersen *et al*., 2001).

Tissue D_5 levels were only high enough to be analyzed using GC/MS in the 160 ppm exposure. Thus, the 160 ppm dose level data were used for parameter estimation. After the model was developed using the 160 ppm exposure data, it was verified that the resulting model was also consistent with key aspects of the 7 ppm exposure, such as percentage retained, amounts appearing in exhaled air, feces, and urine, and the time courses of these processes. Although linked directly through metabolite production by D_5 oxidation, parameters for D_5 and the metabolite models were estimated separately. The use of first-order metabolism for D_5 at the two inhalation concentrations was partially validated by the evaluation of the high concentration exposure for total metabolism and the successful extrapolation of this metabolic clearance term unchanged to the results at 7 ppm.

For the parent D_5 model, 22 parameters were estimated (Table 3). Tissue:blood PCs for the fat, liver and rapidly and slowly perfused tissues were calculated from the ratio of the *in vitro* blood:air and tissue:air PCs, reducing the number of parameters to be estimated from inhalation data. The remaining 18 parameters were estimated by comparing model output to experimental data. Nine data sets from the single and repeat exposures were used to estimate these parameters, focusing on time course data for the concentration of D_5 in the plasma, liver, lung, and fat following single and repeat exposures. Additionally, the rate of D_5 exhalation following completion of the 6 hour single inhalation exposure (measured by adsorption on charcoal) provided information about the circulating concentration of free D_5 available for exhalation. The body burden (based on total radioactivity) of D_5 measured immediately after the exposure ended and the cumulative amount of metabolites in the urine and feces at 168 h following a single inhalation exposure were used for estimation of metabolism and excretion parameters.

For the metabolite model, 12 additional parameters were estimated using the 160-ppm single inhalation exposure data (Table 4). The six data sets used to estimate these parameters included time course data for the concentration of total parent D_5 and metabolites (i.e., estimated by comparison of total radioactivity and measured D_5 in the same samples) in the plasma, liver, lung and fat, the cumulative amount of the metabolite excreted in the feces and the estimates of elimination of degradation products of the hydroxylated cyclic metabolites in the urine.

TABLE 1
Chemical and Partitioning Properties of D_5

	D_5
CAS number	541-02-6
Molecular formula	$C_{10}H_{30}O_5Si_5$
Molecular weight, Da	370.8
Melting point, °C	−38
Vapor pressure at 25°C, mmHg	0.2
In vivo blood:air PC for rats	0.26[a]
In vitro whole blood:air PC for rats	0.72 ± 0.20
In vitro perirenal fat:air PC for rats	1436 ± 325
In vitro liver:air PC for rats	11.1 ± 5.8
In vitro kidney:air PC for rats	3.4 ± 1.3
In vitro muscle:air PC for rats	19.7 ± 14.0
Corn oil:air PC	1436 ± 23
Olive oil:air PC	2263 ± 294

[a]Plasma:air PC calculated using the rat *in vivo* data (Table 4).

TABLE 2
Physiological Parameters Used in the PBPK Models

		Humans	
Parameter	Rats	Male	Female
Body weight (BW), kg	0.20, 0.13[a]	—[b]	—[b]
Fraction of BW in liver[c,d]	0.037	0.0314	0.0314
Fraction of BW in lung[c,d]	0.012	0.0076	0.0076
Fraction of BW in richly perfused tissue[c]	0.038	0.0424	0.0424
Fraction of BW in slowly perfused tissue[c]	0.714	0.5396	0.4996
Fraction of BW in blood[c,d,e]	0.059	0.059	0.059
Fraction of BW in fat[c,f]	0.05	0.23	0.27
Alveolar ventilation rate, l/h/kg$^{0.75}$	15	—[b]	—[b]
Cardiac output (QC), l/h/kg$^{0.75}$	15	—[b]	—[b]
Fraction of QC to liver	0.2	0.227 (0.142)[g]	0.227 (0.142)[g]
Fraction of QC to richly perfused tissue	0.44	0.472 (0.295)[g]	0.472 (0.295)[g]
Fraction of QC to slowly perfused tissue	0.3	0.249 (0.465)[g]	0.249 (0.465)[g]
Fraction of QC to fat[f]	0.06	0.052 (0.098)[g]	0.052 (0.098)[g]

[a]For male and female rats, initial BWs were 0.199 ± 0.006 kg and 0.128 ± 0.003 kg, respectively, for single D_5 exposures.
[b]See Supplemental data.
[c]The sum of the fraction of BW in all compartments is 0.91 because 9% of the body was assumed to receive minimal blood flow. The specific gravity of all tissues was estimated to be 1.
[d]In the deep plasma compartment and the second liver and lung deep compartments, 2% of the tissue was deep compartment.
[e]The arterial and venous blood contained 35 and 65% of the blood volume, respectively.
[f]The rat model included two fat compartments. The fraction of BW in the diffuse and distributed fat was 0.045 and 0.005, respectively, and the fraction of the QC to the diffuse and distributed fat was 0.054 and 0.006, respectively.
[g]Numbers in parentheses are values during exercise periods. Blood flow rates to the rapidly perfused tissue and liver remained the same but blood flow rates to the fat and slowly perfused tissue increased (i.e., 21 and 79% of the increase in blood flow went to the fat and slowly perfused tissues, respectively).

The formation of a combined pool of short chain linear silanol metabolites in a central compartment and the elimination of these metabolites into the urine were included as the urinary excretion pathway in the model for HO-D_5 (Fig. 1). Parameters for this part of the metabolism model (i.e., a first-order rate constant for metabolism of HO-D_5 to form a pool of short chain linear silanols, a volume of distribution for the storage of this combined metabolite pool, and a rate constant for the elimination of this pool into urine) were estimated using data for the cumulative amount of total metabolites recovered in the urine.

A significant amount of nonparent D_5 radioactivity was present in the feces (Tobin *et al*., 2008). High-performance liquid chromatography analysis of the radioactivity suggested that the non-D_5 peak corresponded to the retention time of the HO-D5. Parameters for describing the fecal excretion of the metabolite (i.e., a rate constant for elimination from liver into bile/feces and a delay for the appearance in feces) were estimated from the cumulative excretion in feces. Model simulations could only be compared with data from the single exposure to 160 ppm ^{14}C-D_5 because the amount of parent compound in feces was not determined for the 7 ppm exposure.

Model parameters were calculated separately for male and female rats to determine if similar parameters described the pharmacokinetics for both genders. The model parameters used in all simulations shown in the various figures were determined by taking the average value of model parameters for male and female rats (Tables 3 and 4). This method was used so that as much data as possible went into estimation of model parameters. Only the data for female rats are included in the resultant plots. Male rat data were included in the analysis, but simulation results for male rats are only included in the tabulated results (Tobin *et al*., 2008).

Parameterization of the human model. For the human PBPK model, a variety of physiological parameters were experimentally measured or calculated for each subject (Supplemental Materials, S-7). The effects of exercise were incorporated by varying the alveolar ventilation rate, QP, the cardiac output, QC, and the blood flow rates to two tissue compartments (Table 2). During model-simulated exercise, the blood flow rate to the slowly perfused tissue compartment, which includes muscle tissue, increased. Additionally, the blood flow rate to the fat compartment increased during exercise in a manner consistent with the study of Bulow and Madsen (1978). The blood flow rates to the liver and rapidly perfused tissue remained unchanged from resting levels during the exercise periods.

Six parameters from the rat PBPK model were used in the human model. Human studies with D_4 utilized ^{14}C-D_4 and provided information on production and excretion of silanols over time. With D_5, the compound used was unlabeled, so there were no data on the rate or extent of metabolism in humans. For this reason, the allometric scaling constant for hepatic clearance of D_5 was taken from the rat D_5 PBPK model. Tissue:blood PCs of rats and humans are expected to be similar because tissue compositions are similar, and so tissue:blood PCs for the fat, liver, lung, and rapidly and slowly perfused tissue compartments from the rat model were used in the human model to decrease the number of parameters estimated using experimental data. The value of the blood:air PC, Pb, for individual subjects was estimated from the plasma time course curves. For compounds with low blood:air partitioning, achieved blood concentration is highly sensitive to Pb. Thus, time course data from the human inhalation pharmacokinetic experiment (i.e., the cumulative amount of D_5 absorbed and D_5 plasma concentrations) were used to estimate six parameters including Pb (Table 5).

Model calculations. Two different software packages, Berkeley Madonna (University of California, Berkeley, CA) and ACSL (Aegis Technologies, Dallas, TX), were used to solve model equations. The Supplemental Material section has the equations for the D_5 rat inhalation PBPK model. Model code for both the rats and human models, showing all equations, is available from the corresponding author upon request. Parameters were estimated using the

TABLE 3
Additional Parameters Used in the D_5 Rat PBPK Model[a]

Parameter	Male	Female	Mean
Blood:air PC (Pb)	0.24	0.28	0.26
Fat:blood PC	2000[b]	2000[b]	2000[b]
Liver:blood PC	15[b]	15[b]	15[b]
Lung:blood PC (Plu)	62	64	63
Rapidly perfused tissue:blood PC	4.7[b]	4.7[b]	4.7[b]
Slowly perfused tissue:blood PC	27[b]	27[b]	27[b]
Allometric scaling constant for metabolic clearance in liver, kfc, ml/h/kg$^{0.7}$[c]	2100	1400	1800
Rate constant for hepatic production of mobile lipid pool of D_5 (Kmlp), 1/h	3.9	2.7	3.3
Clearance of unavailable D_5 in mobile lipid pool to fat (CLmlp), ml/h	3.8	3.7	3.8
Slowly perfused tissue uptake clearance, ml/h[d]	0.12QS	0.18QS	0.15QS
Diffuse fat uptake clearance, ml/h[d]	0.03QF1	0.05QF1	0.04QF1
Distributed fat uptake clearance, ml/h[d]	0.04QF2	0.06QF2	0.05QF2
Parameter for transport from liver to deep liver comp. 1, 1/h	1.3	1.7	1.5
Parameter for transport from deep liver comp. 1 to liver, 1/h	0.28	0.42	0.35
Parameter for transport into and out of deep liver comp. 2, ml/h	0.10	0.40	0.25
Deep liver 2:liver PC	500	1300	900
Parameter for transport from lung into deep lung comp. 1 (Klud1), 1/h	0.19	0.090	0.14
Parameter for transport from deep lung comp. 1 into lung (Kdlu1), 1/h	0.24	0.32	0.28
Parameter for transport into and out of deep lung comp. 2 (Klud2), ml/h	0.16	0.14	0.15
Deep lung 2:lung PC (Pdlu)	1200	1100	1200
Parameter for transport into and out of deep blood (Kbld), ml/h	—[e]	0.025	0.025
Deep plasma:plasma PC (Pdb)	—[e]	85	85

[a]Unless otherwise noted, these parameters were estimated by comparing model output to appropriate data sets.
[b]Calculated by dividing *in vitro* measurements of the blood:air PCs by the tissue:air PCs (see Table 1).
[c]Metabolic clearance in the liver is calculated as KFCxBW$^{0.7}$.
[d]The uptake clearance of the slowly perfused compartment and the fat compartments were calculated as a fraction of blood flow to the slowly perfused tissue, QS, diffuse fat, QF1, and distributed fat, QF2, compartments.
[e]For male rats, there were no data sensitive to this parameter.

multiple curve-fitting routine in Berkeley Madonna, which minimizes the root mean square deviation between the data points and the model output. Multiple data sets were used for parameter estimation and so the residuals from each data set were weighted by the inverse of the standard deviation (SD) of the dataset. Parameter estimation approaches for the rat and human was done as described by Reddy *et al.* (2003) for D_4. Parameter estimation was preceded by evaluations of log normalized sensitivity coefficients (LNSC) (Clewell *et al.*, 1994) to insure that data sets chosen for parameter estimation (i.e., for fitting model output to specific data) were sensitive to changes in parameter values. The methodology for LNSC determinations is included in Supplemental Materials **(S-8)**.

RESULTS AND DISCUSSION

D_5 Time Courses in the Rat

The D_5 inhalation PBPK model in the rat could be successfully parameterized to fit tissue concentrations and rates of exhalation for the single exposure (Fig. 2) and tissue concentrations for the repeat exposure (Fig. 3) to 160 ppm ^{14}C-D_5. Because Pb had the largest effect on model output, it was the first parameter to be estimated in the curve-fitting procedures. The body burden of D_5 resulting from a single 6-h exposure, plasma concentrations, and the rate of D_5 exhalation were all highly sensitive to Pb. As noted with D_4, the *in vivo* estimate of Pb is lower than the value estimated from *in vitro* determinations with fresh whole blood (Table 1). After an initial estimate of Pb was obtained, the second parameter estimated was the first-order rate constant for metabolic clearance in liver, kfc. The value of kfc was adjusted so that the total amount of metabolite produced matched the cumulative amount of metabolites recovered from the urine and feces during 168-h postexposure.

For the blood compartment, parameters for the pool of unavailable D_5 that was formed in the liver, transported in the blood and cleared into the fat (i.e., for the model structure simulating the movement of lipoprotein transport of bound D_5) heavily influenced blood concentrations for the 24-h period following the single and repeat inhalation exposures. Without the inclusion of this pool of D_5 in the blood, a much higher value of Pb would be required to describe blood concentration; however, this option leads to a gross overestimation of body burden. To describe blood concentrations in female rats at extended times following the repeat exposure, a deep tissue compartment was required in the blood for females. This compartment was not required in describing kinetics in the male. The sensitivity of predicted blood concentrations to appropriate model parameters at several times—Supplemental

TABLE 4
Additional Parameters Used in the Rat PBPK Model for HO-D_5[a]

Parameter	Male	Female	Mean
Fat:blood PC	19	22	21
Liver:blood PC	35	35	35
Lung:blood PC	53	35	44
Rapidly perfused tissue:blood PC	35[b]	35[b]	35[b]
Slowly perfused tissue:blood PC	25	30	28
Allometric scaling constant for metabolism of HO-D_5 to silanols in the liver, ml/h/kg$^{0.7}$[c]	130	110	120
Renal clearance of the combined silanol metabolite pool, ml/h	> 80[d]	> 80[d]	> 80[d]
Scaling constant for the volume of distribution of the combined metabolite pool, ml/mg[e]	< 1[d]	< 1[d]	< 1[d]
Clearance of HO-D_5 into feces, ml/h	23	28	26
Delay for fecal excretion, h	12	12	12
Fat uptake clearance, ml/h[f]	0.1QF	0.1QF	0.1QF
Parameter for transport from lung into deep lung, 1/h	0.008	0.006	0.007
Parameter for transport from the deep lung into the lung, 1/h	0.0005	0.0005	0.0005

[a]Unless otherwise noted, these parameters were estimated by comparing model output to appropriate data sets.
[b]This value was set to the liver:blood PC.
[c]Metabolic clearance in the liver was calculated by multiplying the allometric scaling constant by BW$^{0.7}$.
[d]Model output was not sensitive to the exact value of these parameters. They have to be set so that the combined metabolite pool clears rapidly from the blood into the urine.
[e]The volume of distribution for the combined metabolite pool was calculated by multiplying the scaling constant by BW.
[f]The uptake clearance of the fat compartment was calculated as a fraction of blood flow to the fat compartment, QF.

Materials (S-9)—indicated how different parts of the time course curves can be used in estimating different model parameters. This differential sensitivity was especially important in estimating parameters for uptake into deep compartments within tissues.

Because simple flow-limited uptake by the lung and liver compartments underestimated these tissue concentrations, a single deep compartment was initially added to these tissues to improve model fits with D_5 concentrations in these tissues. However, a single deep compartment could not be parameterized to describe both the single and the repeated exposures studies with common compartment characteristics. For the D_5 PBPK model to successfully describe the single and repeat exposures, two deep compartments were included in each of these tissues. The first deep compartment had a larger contribution to D_5 concentrations in the lung and liver for 24-h postexposure. The second deep compartment provided the ability to account for the tissue concentrations after the repeat exposure. The need for these deep compartments indicates that these tissues contain several kinetically distinct pools of D_5; these additional pools are likely related to the high lipophilicity of D_5 with concomitant high solubility in various lipid structures within tissues/cells. The differences in tissue deep stores between D_4 and D_5 may is likely due to fat:blood partitioning with D_5 that accentuates storage in cellular lipids for D_5. The fat:blood PC for D_5 and D_4 in the models were 2000 (Table 3) and 550 (Andersen *et al.*, 2001), respectively.

Successful fitting of the exhaled air and blood siloxane, required a pool of blood D_5 that was not in equilibrium with free D_5 in blood. This pool is different from a deep compartment that equilibrates through free blood D_5. Although the nature of this "unavailable" siloxane is not known exactly, the kinetic modeling was based loosely on lipoprotein production and transport. At present, the successful development of these PBPK models with VCMS, indicates that the relationship between free tissue or blood siloxane and total siloxane in the tissues is also complexly related to exposure history.

In female rats, the time-averaged D_5 fat tissue concentrations following the single and repeat exposures were about 3 and 190 μg/ml, respectively (i.e., approximately the same as in

TABLE 5
Additional Parameters Used in the Human PBPK Model[a]

	Subject no.					
Parameters	1	2	3	4	5	Average[b]
Pb	0.56	0.30	0.43	0.27	0.51	0.41 ± 0.13
Kdlu, 1/min	0.0014	0.0015	0.00082	0.0045	0.0099	0.0036 ± 0.0038
Kld, 1/min	0.072	0.0089	0.030	0.090	0.16	0.072 ± 0.059
Klud, l/min	0.064	0.21	0.054	0.15	0.12	0.12 ± 0.06
Kmlp, 1/min	0.078	0.037	0.044	0.12	0.10	0.076 ± 0.036
CLmlp, l/min	0.036	0.068	0.021	0.081	0.024	0.046 ± 0.027

[a]Tissue:blood PCs for the lung, liver, fat, and rapidly and slowly perfused compartments and KFC were taken from the rat model (see Table 4).
[b]Average for five subjects ± 1 SD.

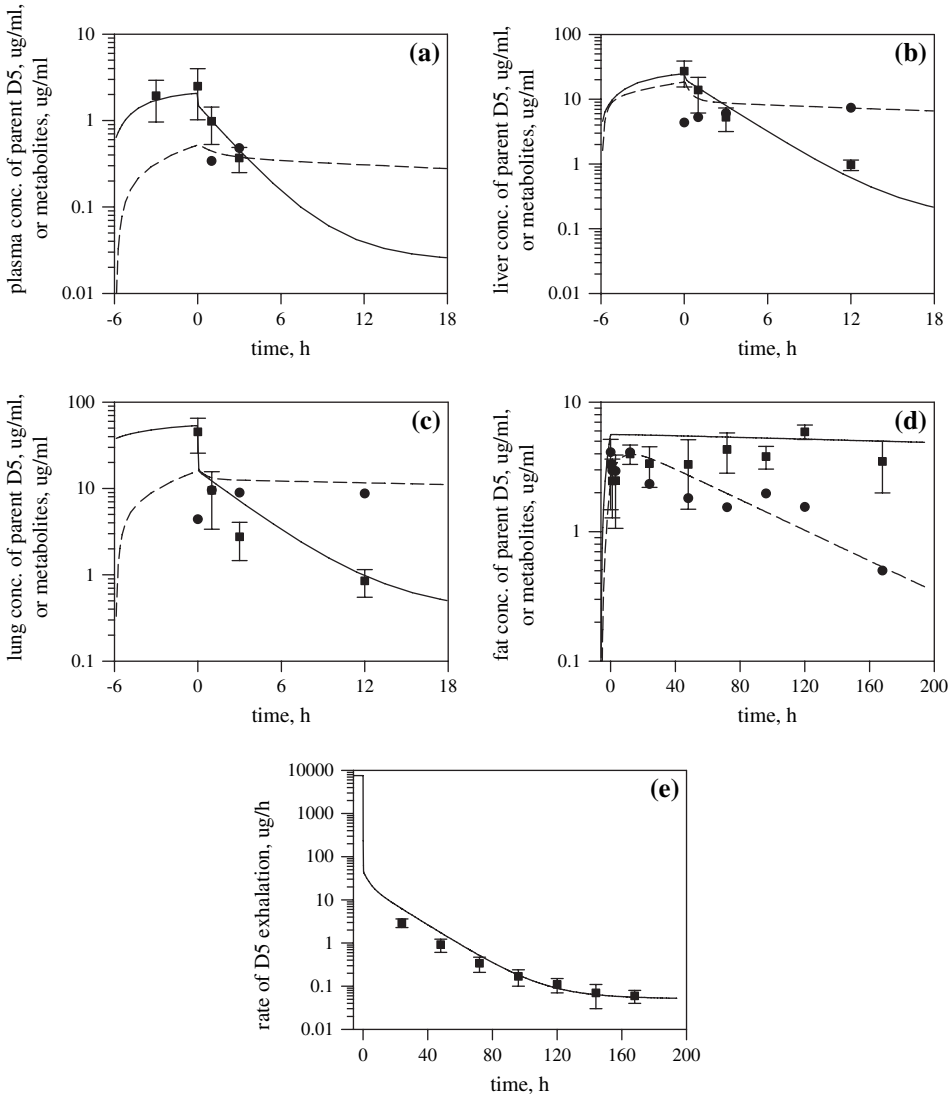

FIG. 2. Measured and calculated parent D_5 (closed square, solid curves) and metabolite (closed circle, dashed curves) concentrations in the (a) plasma, (b) liver, (c) lung, (d) fat, and (e) the rate of D_5 exhalation in female rats exposed to a single exposure of 160 ppm ^{14}C-D_5 by inhalation. Error bars for D_5 measurements represent one SD for three to five rats. For the model structure presented here, metabolite radioactivity in the liver, lung, and fat tissue was assumed to be a single metabolite. In fat, metabolite radioactivity was assumed to be HO-D_5. In the plasma a mixture of metabolites was assumed. Data are from Tobin *et al.* (2008).

male rats for the single exposure, but higher than in male rats for the repeat exposure). In male rats, the fat concentration was about 24 times higher following a 15-day exposure than following a single exposure (73 vs. 3 μg/ml), but in female rats the fat concentration was about 63 times higher following a 15-day exposure than following a single exposure (190 μg/ml/3 μg/ml). Although the fat concentrations in male rats were well described for both the single and repeat exposures (data not shown), the concentrations of D_5 in fat in female rats were overestimated for the single exposure (Fig. 2) and underestimated for the repeat exposure (Fig. 3). With D_5, gender differences were apparent in relation to both fat tissue uptake and the need for a deep compartment within the plasma/blood. Gender differences were not noted in the kinetics in fat with D_4 and the physiological basis for these gender related differences in tissue storage of D_5 in rats is not known.

The time course behaviors inferred for the fat compartment were consistent with diffusion-limited uptake. Its inclusion of diffusion-limited uptake for the slowly perfused tissues improved fits to both the body burden and the rate of D_5 exhalation. The slowly perfused tissues represent the largest proportion of the body weight of the rat. With flow-limited uptake, this compartment filled too rapidly leading to overestimates of total body burden. The slower tissue uptake for the more lipophilic compound, D_5 compared with D_4, is likely due to the difficulties of transferring the siloxanes from an

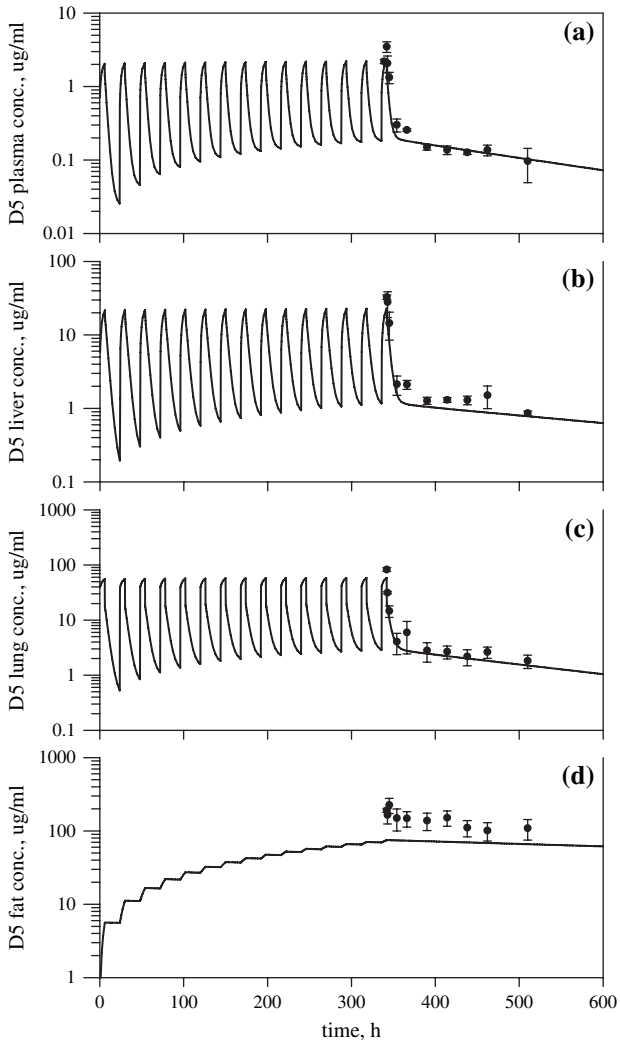

FIG. 3. Measured and calculated Parent D_5 concentrations in the (a) plasma, (b) liver, (c) lung, and (d) fat of female rats following a repeated 6-h inhalation exposure to 160 ppm for 14 days followed by a 6-h exposure to ^{14}C-D_5 on day 15. Error bars represent one SD for $n = 4$ or 5. Data are from Tobin *et al.* (2008). Fat concentrations in male rats were considerably lower and the best-fit constants were developed considering both data sets, producing lower predictions for the female rat fat compared with the measured data.

essentially aqueous milieu in blood to move across the lipid membrane of cells and into a more varied tissue environment.

Stable Metabolite Kinetics, Distribution, and Elimination

The tissue compartments in the HO-D_5 model structure were similar to those used for D_5, although there was only one fat compartment. Fat tissue uptake of the metabolite was still described as diffusion-limited and to describe time course data of radioactivity attributed to metabolites in lung tissue, a deep compartment was required in the lung.

Our modeling efforts calculated concentrations of D_5, the HO-D_5, and a combined pool of linear silanols in tissues, blood and excreta over time. Total tissue radioactivity, as modeled, consisted of two components—D_5 and the HO-D_5. This assumption was confirmed for the fat (Tobin *et al.*, 2008). However, in other tissues, such as the liver and lung, linear silanols may account for some of the radioactivity. Blood radioactivity was described as a combination of D_5, HO-D_5 and the silanols. By combining these three models, it was possible to simulate total tissue radioactivity (D_5 and metabolites) following the single 7 and 160 ppm exposures. Although the parameters for D_5 and metabolites were estimated using data from the 160 ppm exposures, simulations of tissue time courses for the 7 ppm exposures were in good agreement with the experimental results for total tissue radioactivity (Fig. 4) and for cumulative elimination in urine and feces (Fig. 5). However, the body burden estimates for the 7 ppm ^{14}C-D_5 exposures were slightly overestimated in these rats. The body burden dose calculated using the D_5 PBPK model was 99 and 70 μg eq D_5 for male and females, respectively. The body burdens estimated by experimental determinations of the carcass and tissues were 78 and 34 μg eq D_5 for male and female rats, respectively.

At times where the tissue concentrations of the metabolites could be estimated, the liver and lung tissue concentrations were higher than in fat (Fig. 2). For example, in female rats 12 h after a 160 ppm ^{14}C-D_5 exposure had ended, the radioactivity concentrations not attributed to D_5 in the liver, lung and fat tissue were 7.4, 8.7 and 4.1 μg eq D_5/ml, respectively. PCs for the HO-D_5 in various tissues were estimated from the time course data and PK analyses (Table 4). The estimated fat:blood PC (21) was lower than the liver:blood PC (35) or the lung:blood PC (44). The larger PCs for liver and lung compared with the fat were unexpected for a compound that should still be highly lipophilic. This discrepancy is likely due to treating all the metabolite radioactivity in the liver and lung as the HO-D_5. Linear silanols may account for some portion of the radioactivity in liver and lung.

Accounting for D_5 Kinetics in Humans

The goal in developing the model structure for humans was to create the simplest model structure that remains consistent with the presence of blood D_5 in humans even after exhalation rates fall significantly. The simulated curves were calculated using the average values of the inhaled D_5 concentration and physiological properties for males and females (Table 2 and Supplemental Materials S-10) and the average values of estimated model parameters (Table 5). As with the rat, the human PBPK model had a deep compartment in the lungs, a deep compartment in the liver, and a pool of sequestered D_5 in the blood. Because the uptake of D_5 by the fat tissue in the rat is diffusion-limited, fat tissue uptake of D_5 in the human is also likely to be diffusion-limited. However, the parameter for diffusion-limited uptake in humans could not be calculated because none of the available human pharmacokinetic data over the 24-h observation period were sufficiently sensitive to the properties of the fat compartment. For the sake of

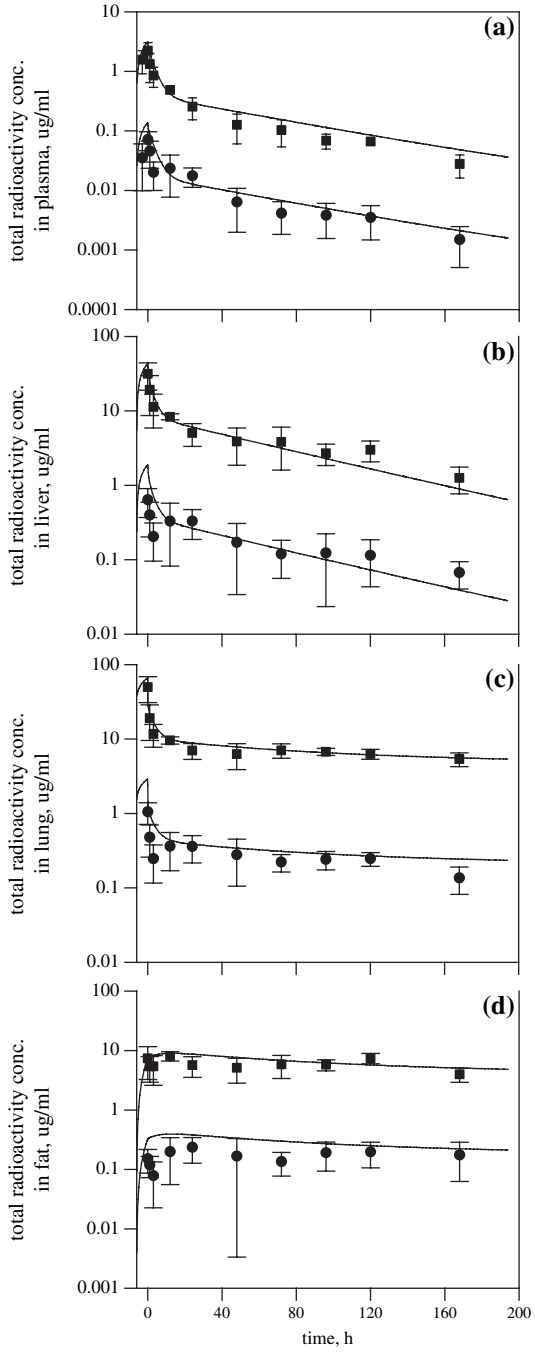

FIG. 4. Measured and calculated total radioactivity concentrations in the (a) plasma, (b) liver, (c) lung, and (d) fat of female rats exposed to a single exposure of 160 ppm (closed square) and 7 ppm (closed circle) ^{14}C-D_5 for 6 h. Error bars represent one SD for $n = 4$ or 5. Data are from Tobin *et al*. (2008).

parsimony, uptake of D_5 by fat in humans was described as blood flow-limited. Uptake of D_5 by the slowly perfused compartment was also described as perfusion-limited in humans. This model structure accurately accounted for the time course behavior and the amount retained, measured as the summation of inhaled versus exhaled differences.

In general the human inhalation PBPK model for D_5 described the concentration of D_5 in exhaled air and plasma (Fig. 6). Simulated D_5 exhaled air concentrations increased between 10–20 min and 40–50 min (i.e., during the exercise periods) due to physiological changes from exercise that were incorporated in the model. Although model calculations are presented for average parameter values, parameters calculated for each individual subject are reported to illustrate the interindividual variability in parameter values (Table 5). Some interindividual variability in D_5 pharmacokinetics was noted; D_5 plasma concentrations of individual subjects 24 h after the 1-h exposure varied by 10-fold (Fig. 6). The individual estimates of Pb only varied by a factor of 2 (0.27–0.56). Thus, the postexposure differences in plasma concentrations are primarily related to differences in uptake of D_5 into the nonavailable bound pool. This human PBPK model was also used to estimate the amounts of D_5 eliminated by exhalation and metabolism (Table 6).

Controlling Free Concentrations of VCMS

Clearance of free D_5 from the circulation is largely due to exhalation and metabolic clearance. Pb for D_5 in humans was about 0.4 (Table 5) and in rats was about 0.3 (Table 3). For compounds with such low values of Pb, exhalation is a major pathway of elimination. In the postexposure period, clearance by exhalation, CLex, for free D_5 can be readily calculated from physiological parameters (Reddy *et al*., 2003). For humans and animals at rest with approximately equal cardiac output and alveolar ventilation the CLex is approximated by the ratio $1/(1 + Pb)$. This ratio is 0.7 QC in humans and about 0.8 QC in rats. Hepatic extraction, defined as the hepatic clearance divided by the sum of the hepatic clearance and the blood flow to the liver, is about 0.39 in rats and nearly as high in humans. With a hepatic extraction of 0.4, the systemic extraction due to metabolism would be 0.4 times the proportion blood flow to liver - 0.2 QC (Table 2). Thus, the hepatic clearance is 0.08 QC, or about 10% of exhalation clearance. The behavior of these VCMS is very much distinct from most VOCs. The high fat partitioning leads to slow rates of uptake into tissues and slow loss from tissues, especially fat after cessation of exposures. However, free concentrations fall rapidly, within minutes, after cessation of an inhalation exposure.

With such a large fat:blood PC there could be concern of bioaccumulation of D_5 in exposed individuals. However, the main characteristic for bioaccumulation of nonvolatile compounds is low daily clearances not lipophilicity, as noted in PBPK models for polychlorinated biphenyls (Anderson *et al*., 1977) or dioxin (Carrier *et al*., 1995). With D_5 it is more correct to talk about high lipophilicity and a tendency to be present at concentrations higher in fat than in blood or other tissues with slower approach to steady-state tissues exposures during repeated, episodic exposures. Unlike the situation with bioaccumulative compounds, such as persistent PCBS or

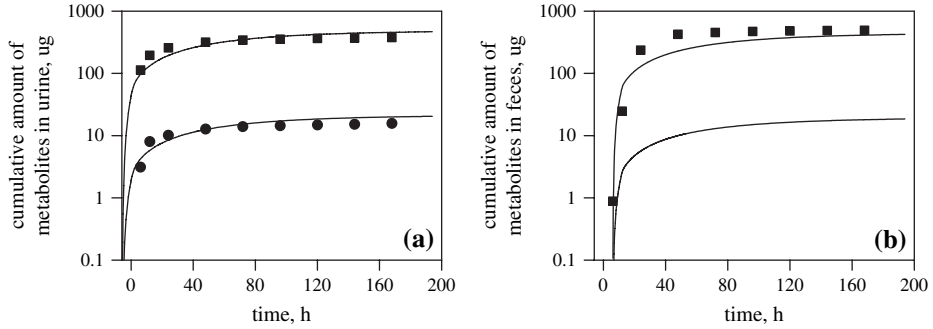

FIG. 5. Measured and calculated cumulative (a) metabolites excreted in the urine and (b) the feces of female rats exposed to 7 ppm (closed circle) and 160 ppm (closed square) ^{14}C-D_5 by inhalation for 6 h. Data are from Tobin *et al.* (2008).

perfluoroacids, significant increases in free concentrations in blood/plasma or in central compartments do not occur with repeated exposures of these VCMS (Andersen *et al.*, 2008). The ability to confidently estimate free concentrations in blood, plasma, and tissues is essential for equating biological outcomes with appropriate measures of dose, for conducting dosimetry based risk assessments, and, with these VCMS, for evaluating whether they should be regarded as having a potential for bioaccumulation in air breathing species.

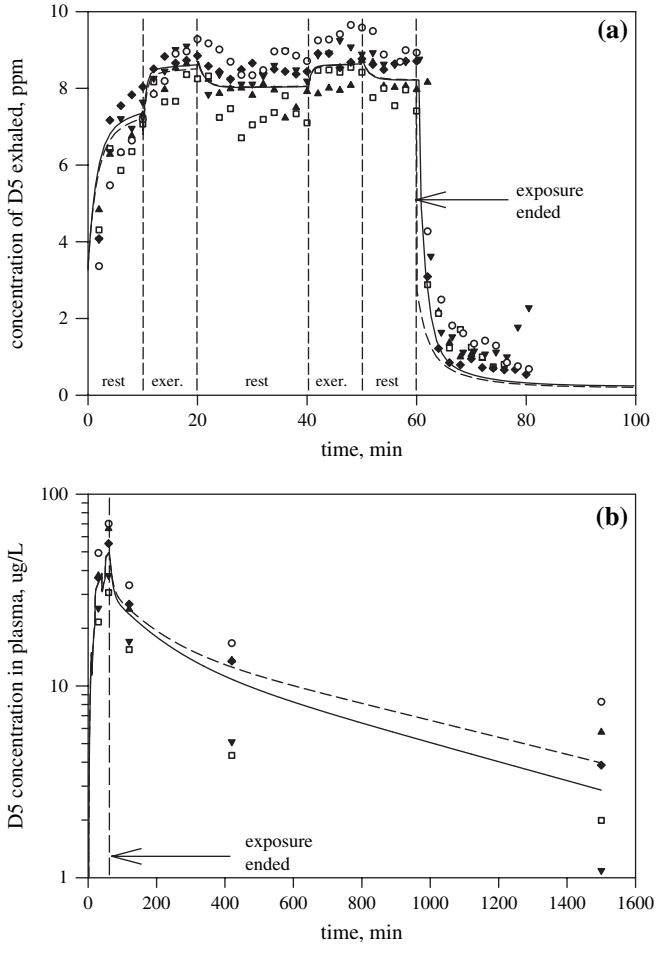

FIG. 6. D_5 concentrations in (a) exhaled air and (b) plasma as a function of time in women (open symbols, dashed line) and men (closed symbols, solid line) exposed to 10 ppm D_5 by inhalation. The curves were calculated using best-fit parameter values from Table 5.

Summary

Modeling the pharmacokinetics of inhaled D_5 in the rat was greatly aided by previous modeling with D_4. The D_5 rat PBPK model included parent compound, a stable cyclic metabolite, and a pool of silanols. After successful application in describing the broad data set on D_5 in rats, a similar, though simplified, PBPK model structure was used then to describe kinetic results obtained in exposures to five human volunteers. Like D_4, D_5 has the unusual combination of low blood:air and high fat:blood partitioning, giving rise to preferential storage in lipid compartments in the body, including some sequestration in blood in a bound unavailable pool. A consistent PBPK model structure, with similar PCs and compartmental structures

TABLE 6
Summary of Results for D_5 Human Inhalation Exposures

	Experimental data		Model calculations	
Measured quantity	Women[a]	Men[b]	Women	Men
Amount retained during the 60-min exposure, mg	24.0[a]	24.9 ± 2.0[b]	25.8	28.1
Cumulative amount of D_5 exhaled 1 day after the exposure, mg	—[c]	—[c]	17.5	21.0
Cumulative amount of D_5 metabolized during the 1-h exposure, mg	—[c]	—[c]	0.5	0.5
Cumulative amount of D_5 metabolized 1-day postexposure, mg	—[c]	—[c]	1.9	1.6

[a]Average value for two subjects.
[b]Average values ± 1 SD for three subjects.
[c]This could not be calculated using the experimental data.

described D_5 pharmacokinetics in rats and humans and can be used in predicting time courses of free D_5 in both species. The conclusion reached using PBPK approaches to analyze time course kinetic data with VCMS counsel caution in simply measuring VCMS concentrations in biological media and automatically assuming that all measured compound is available for interactions with possible targets within tissues.

SUPPLEMENTARY DATA

Supplementary data are available online at http://toxsci.oxfordjournals.org/.

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