Physiological Modeling of Inhalation Kinetics of Octamethylcyclotetrasiloxane in Humans during Rest and Exercise

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In a recent pharmacokinetic study, six human volunteers were exposed by inhalation to 10 ppm 14C-D4 for 1 h during alternating periods of rest and exercise. Octamethylcyclotetrasiloxane (D4) concentrations were determined in exhaled breath and blood. Total metabolite concentrations were estimated in blood, while the amounts of individual metabolites were measured in urine. Here, we use these data to develop a physiologically based pharmacokinetic (PBPK) model for D4 in humans. Consistent with PBPK modeling efforts for D4 in the rat, a conventional inhalation PBPK model assuming flow-limited tissue uptake failed to adequately describe these data. A refined model with sequestered D4 in blood, diffusion-limited tissue uptake, and an explicit pathway for D4 metabolism to short-chain linear siloxanes successfully described all data. Hepatic extraction in these volunteers, calculated from model parameters, was 0.65 to 0.8, i.e., hepatic clearance was nearly flow-limited. The decreased retention of inhaled D4 seen in humans during periods of exercise was explained by altered ventilation/perfusion characteristics during exercise and a rapid approach to steady-state conditions. The urinary time course excrution of metabolites was consistent with a metabolic scheme in which sequential hydrolysis of linear siloxanes followed oxidative demethylation and ring opening. The unusual properties of D4 (high lipophilicity coupled with high hepatic and exhalation clearance) lead to rapid decreases in free D4 in blood. The success of D4 PBPK models with a similar physiological structure in both humans and rats increases confidence in the utility of the model for predicting human tissue concentrations of D4 and metabolites during inhalation exposures.

Key Words: octamethylcyclotetrasiloxane; D4; inhalation pharmacokinetics; PBPK modeling; vapor retention; flow-limited metabolism; lipid sequestration.

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related to circulating lipids (Andersen et al., 2001); however, studies have not been done to track D₄ in blood lipids over time after an inhalation exposure.

Because of the low order of D₄ toxicity, two pharmacokinetic studies of D₄ inhalation in humans have been performed in which volunteers were exposed to 10 ppm D₄ during alternating periods of rest and exercise. In the more comprehensive of the two studies, ¹⁴C-D₄ was administered and the concentration of parent compound in the blood and exhaled breath and the amount of metabolite in the blood and urine were determined (Utell, 2000). In this article we describe a PBPK model developed for D₄ inhalation exposures in humans using these pharmacokinetic data. Adopting the strategy of Andersen et al. (2001), we first developed a simple PBPK model for human inhalation exposures to D₄ and added additional components as necessary. The resulting refined human PBPK model that successfully described the data with the radiolabeled D₄ was then validated by successfully predicting blood and exhaled breath levels for an earlier pharmacokinetic study of D₄ in human volunteers conducted with unlabelled D₄ (Utell et al., 1998). Our success in developing a consistent PBPK model with common physiological structure for both rats and humans indicates that this model should be useful for assessing tissue exposures from inhaled D₄ in humans for a variety of exposure situations and supporting human risk assessments for this compound.

**MATERIALS AND METHODS**

**Experimental data.** The pharmacokinetic study on the inhalation exposure of humans to D₄ (Utell, 2000) was completed at the University of Rochester following the approval of the Human Subject Review Committee. During a 1-h exposure to 10 ppm ¹⁴C-D₄ using a mouthpiece exposure system described previously (Utell et al., 1998), six male volunteers rested for 10 min, exercised for 10 min, rested for 20 min, exercised for 10 min, and then rested for 10 min. For exercise, the subjects barked at a resistance that tripled their resting minute ventilation. For each subject, the respiration rate and minute volume were determined during the exposure (Table 1). The D₄ concentration in the inhaled and exhaled air was monitored regularly during the exposure, and the exhaled concentration was monitored during a 20 to 30-min postexposure period. Samples of expired breath were also obtained at 1, 3, 6, 24, 48, and 72 h after the exposure ended by the collection of end-expiratory air in a 40-l Tedlar bag. Blood samples were drawn from the forearms of each subject at 0, 30, 60, 120, 240, 420, and 1500 min after the exposure began. At the end of the 30-min postexposure period, a spot urine sample was collected and each subject emptied his bladder. For the remainder of the 24 h following the exposure, subjects collected urine in three 8-h aliquots. After the first 24 h, spot urine samples were collected once a day for up to seven days and adjusted for the total amount eliminated based on the amount of creatinine in the urine samples.

**TABLE 1**

**Physiological Parameters and Exposure Concentrations for Subjects of Two Studies**

<table>
<thead>
<tr>
<th>Period</th>
<th>Subj. 1</th>
<th>Subj. 2</th>
<th>Subj. 3</th>
<th>Subj. 4</th>
<th>Subj. 5</th>
<th>Subj. 6</th>
<th>Average</th>
<th>Men (average)*</th>
<th>Women (average)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>52</td>
<td>51</td>
<td>34</td>
<td>35</td>
<td>24</td>
<td>42</td>
<td>39.7 ± 10.8</td>
<td>32.4 ± 9.5</td>
<td>35.5 ± 4.0</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>91</td>
<td>74</td>
<td>75</td>
<td>76</td>
<td>75</td>
<td>83</td>
<td>79.0 ± 6.7</td>
<td>70.9 ± 10.9</td>
<td>69.5 ± 9.7</td>
</tr>
<tr>
<td>Exposure conc. (ppm)</td>
<td>10.4</td>
<td>10.3</td>
<td>10.0</td>
<td>9.96</td>
<td>8.89</td>
<td>8.89</td>
<td>9.56 ± 1.01</td>
<td>10.3 ± 0.4</td>
<td>9.75 ± 0.51</td>
</tr>
<tr>
<td>Average tidal volume(l)</td>
<td>Rest 1</td>
<td>0.76</td>
<td>0.61</td>
<td>0.73</td>
<td>0.74</td>
<td>0.73</td>
<td>0.76 ± 0.12</td>
<td>0.71 ± 0.15</td>
<td>0.63 ± 0.11</td>
</tr>
<tr>
<td>Dead space (l)</td>
<td>0.25</td>
<td>0.20</td>
<td>0.24</td>
<td>0.25</td>
<td>0.24</td>
<td>0.23</td>
<td>0.25 ± 0.04</td>
<td>0.24 ± 0.05</td>
<td>0.21 ± 0.04</td>
</tr>
<tr>
<td>Average minute volume(l/min)</td>
<td>Rest 1</td>
<td>11.8</td>
<td>9.64</td>
<td>12.0</td>
<td>11.5</td>
<td>10.6</td>
<td>12.9</td>
<td>11.4 ± 1.1</td>
<td>11.2 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>Exer. 1</td>
<td>38.3</td>
<td>22.1</td>
<td>39.9</td>
<td>38.1</td>
<td>39.1</td>
<td>51.6</td>
<td>38.2 ± 9.4</td>
<td>34.8 ± 4.2</td>
</tr>
<tr>
<td></td>
<td>Rest 2</td>
<td>15.4</td>
<td>11.7</td>
<td>14.7</td>
<td>14.6</td>
<td>8.47</td>
<td>20.2</td>
<td>14.2 ± 3.9</td>
<td>13.2 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>Exer. 2</td>
<td>42.6</td>
<td>25.2</td>
<td>39.4</td>
<td>35.0</td>
<td>29.3</td>
<td>59.1</td>
<td>38.4 ± 12.0</td>
<td>32.9 ± 3.9</td>
</tr>
<tr>
<td></td>
<td>Rest 3</td>
<td>22.1</td>
<td>12.4</td>
<td>17.1</td>
<td>16.0</td>
<td>11.3</td>
<td>24.6</td>
<td>17.3 ± 5.3</td>
<td>14.8 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>Postexp.</td>
<td>16.1</td>
<td>11.1</td>
<td>11.6</td>
<td>11.5</td>
<td>7.72</td>
<td>14.9</td>
<td>12.2 ± 3.0</td>
<td>11.3 ± 1.3</td>
</tr>
<tr>
<td>Average QP (l/min)</td>
<td>Rest 1</td>
<td>7.83</td>
<td>6.45</td>
<td>8.01</td>
<td>7.69</td>
<td>7.04</td>
<td>8.57</td>
<td>7.6 ± 0.7</td>
<td>7.4 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>Exer. 1</td>
<td>33.5</td>
<td>18.7</td>
<td>35.1</td>
<td>33.0</td>
<td>32.3</td>
<td>43.7</td>
<td>32.7 ± 8.0</td>
<td>29.7 ± 3.5</td>
</tr>
<tr>
<td></td>
<td>Rest 2</td>
<td>10.9</td>
<td>8.27</td>
<td>10.5</td>
<td>10.6</td>
<td>4.3</td>
<td>14.5</td>
<td>9.9 ± 3.4</td>
<td>9.1 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>Exer. 2</td>
<td>36.8</td>
<td>21.7</td>
<td>34.2</td>
<td>29.6</td>
<td>23.0</td>
<td>49.7</td>
<td>32.5 ± 10.3</td>
<td>27.7 ± 3.4</td>
</tr>
<tr>
<td></td>
<td>Rest 3</td>
<td>16.6</td>
<td>8.97</td>
<td>12.5</td>
<td>11.7</td>
<td>6.33</td>
<td>17.9</td>
<td>12.3 ± 4.4</td>
<td>10.6 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>Postexp.</td>
<td>11.3</td>
<td>7.56</td>
<td>7.55</td>
<td>7.58</td>
<td>3.73</td>
<td>9.08</td>
<td>7.8 ± 2.5</td>
<td>7.5 ± 1.3</td>
</tr>
<tr>
<td>Resting QC (l/min)</td>
<td>5.03</td>
<td>5.08</td>
<td>6.29</td>
<td>6.20</td>
<td>7.33</td>
<td>5.66</td>
<td>5.9 ± 0.9</td>
<td>6.5 ± 0.8</td>
<td>6.2 ± 0.4</td>
</tr>
<tr>
<td>Exercising QC (l/min)</td>
<td>8.09</td>
<td>8.19</td>
<td>10.1</td>
<td>9.99</td>
<td>11.8</td>
<td>9.12</td>
<td>9.6 ± 1.4</td>
<td>10.5 ± 1.3</td>
<td>9.9 ± 0.6</td>
</tr>
</tbody>
</table>

*Average values are reported as mean ± one SD.
†Eight men and four women participated in this study.
‖Estimated as one-third the average tidal volume during the first rest period.
*Calculated as the minute volume minus the dead space times the respiration rate.
*Estimated using the correlation (Brown et al., 1997); QC, l/min = −6.846 log (age, years) + 16.775.
*Estimated as 160% of resting QC.
Several analytical techniques were used to measure D₄ and metabolite concentrations in various samples. During the exposure and 30-min postexposure periods, the analysis of D₄ in the exhaled breath was determined using a gas chromatograph (GC) with a flame ionization detector (FID). Samples of exhaled breath taken after the 30-min postexposure period were passed through an adsorbent for trapping volatiles and then through a CO₂ absorbent to determine the amount of ¹⁴C-CO₂ separately from the radioactivity associated with volatile parent or metabolites. In rats, all radioactivity captured in the volatile trap was parent compound (Plotzke et al., 2000), and the same was expected to be true for humans (i.e., chemical trapped in the volatile trap can be assumed to be parent D₄). Liquid scintillation counting (LSC) was used to determine the amount of ¹⁴C-D₄ and ¹⁴C-CO₂ in samples of exhaled breath collected more than 30 min after the exposure ended. Whole blood and blood plasma samples were analyzed with the LSC and gas chromatography/mass spectrometry (GC/MS, as described by Varaprath et al., 2000) analytical techniques. The amount of total metabolites in the blood can be calculated by subtracting the amount of parent compound measured by GC/MS from the amount of radioactivity measured by LSC (i.e., parent compound and metabolite). Urine samples were analyzed using LSC. Additionally, individual metabolites in urine were separated using high-performance liquid chromatography (HPLC) and quantitatively analyzed using LSC.

**Model structure.** Initially, a fairly conventional PBPK model, as used by Ramsey and Andersen (1984) with styrene, was used to describe D₄ disposition in humans with flow-limited uptake in the fat, liver, and rapidly and slowly perfused tissues, along with separate compartments for the venous and arterial blood (Fig. 1). In the simple model, all the D₄ in the blood was available for equilibration with tissues and lung air. In the rat the rate of metabolism had been modeled by a Michaelis-Menten term in the liver and included provision for induction of metabolism at 700 ppm D₄. Because the human subjects were exposed to only 10 ppm D₄, metabolism was described with dose-independent liver clearance without provision for induction. The conventional model structure also included a one-compartment pharmacokinetic model for a combined metabolite pool in blood.

In the rat, PBPK modeling results indicated that some D₄ in the blood was unavailable for exchange with exhaled air and the amount of D₄ in this pool varied with time (Andersen et al., 2001). The successful model in the rat had deep tissue compartments and a pool of D₄ in blood that was unavailable for exhalation or tissue uptake. A second refined PBPK model was developed with the human data to determine if such an elaboration on the conventional model structure was also necessary to account for human kinetics. The refined model (Fig. 2) included a blood pool of unavailable D₄. As with the rat model, this unavailable pool of D₄ was assumed to be produced in the liver, transported in the blood, and cleared by the fat tissue. This behavior is representative of lipoprotein transport. A mass transfer resistance was required to describe the uptake of D₄ by fat tissue in rats. In the human model we included a mass transfer resistance to uptake in the slowly perfused compartment and a deep storage compartment in fat. The role of these compartments in describing the experimental data is noted in the Results. Model equations are provided in Appendix 1 and the code is available by e-mail from the corresponding author.

**Metabolism.** Time course data for the amount of individual metabolites in the urine were available for the development of a more detailed model of D₄ biotransformation pathways in humans. After determining the kinetic parameters for net metabolic clearance of parent D₄, the data for the amount of individual metabolites in the urine were used to create a more complete description of the formation and clearance of individual metabolites. The proposed metabolic scheme and model structure (Figs. 3 and 4) show the expected interrelationships of the individual metabolites that were included in the model for D₄ metabolism.
Parameterization. A variety of physiological parameters were experimentally measured or calculated for the individual subjects of the study (Table 1). Effects of exercise were incorporated in the model by adjusting the alveolar ventilation rate, QP, the cardiac output, QC, and the blood flow rates to two tissue compartments (Table 2; see Appendix 2 for a listing of the abbreviations used in the models). During exercise, the blood flow rate to the fat compartment increased in a manner consistent with the study of Bulow and Madsen (1978), and the blood flow rate to the slowly perfused tissue compartment, which includes muscle tissue, increased during exercise. The blood flow rates to the liver and rapidly perfused tissue were the same during both the rest and exercise periods.

To minimize the number of parameters to be estimated, values of the liver:blood, fat:blood, and rapidly perfused tissue:blood partition coefficients were calculated by dividing the experimental, in vitro, rat-tissue values for the liver:air, fat:air, and kidney:air partition coefficients, respectively, by the blood:air partition coefficients (Andersen et al., 2001). Tissue partition coefficients of rats and humans are expected to be similar because tissue compositions are similar. Because in vitro partitioning data were not available for the slowly perfused tissue compartment, the partition coefficient for D₄ between the slowly perfused compartment and blood, PS, was set to 3. The value of PS cannot be calculated from the in vivo data because fits of the model to these data (e.g., time course concentrations of D₄ in blood plasma) were not sensitive to the value of PS. This parameter did affect the estimates of the amount of chemical retained during inhalation exposure. Although no experimental data were available for the estimation of PS, the potential effect of error in this parameter is small. The slowly perfused compartment is large, but PS is small compared to the partition coefficients of other compartments, and so it stores a relatively small amount of chemical.

For the simple model, four parameters were estimated from the experimental data: (1) the blood:air partition coefficient (Pb), (2) the allometric scaling constant for metabolic clearance in the liver (KFC), (3) the renal clearance of metabolite into the urine (KEL), and (4) a first-order mass transfer coefficient for the movement of D₄ from the fat compartment into a deep compartment (KFD).

![Figure 3](https://example.com/fig3.png)

**FIG. 3.** Proposed reaction mechanism for D₄ metabolism in humans. *Designates compounds that were detected in urine samples.

**TABLE 2**

<table>
<thead>
<tr>
<th>Parameter Used in the PBPK Models</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fraction of blood flow to tissues</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>0.227 (0.142)*</td>
</tr>
<tr>
<td>Fat</td>
<td>0.052 (0.098)*</td>
</tr>
<tr>
<td>Rapidly perfused tissue</td>
<td>0.472 (0.295)*</td>
</tr>
<tr>
<td>Slowly perfused tissue</td>
<td>0.249 (0.465)*</td>
</tr>
<tr>
<td>Fraction of body weight in tissues</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>0.0314*</td>
</tr>
<tr>
<td>Fat</td>
<td>0.23*</td>
</tr>
<tr>
<td>Rapidly perfused tissue</td>
<td>0.05*</td>
</tr>
<tr>
<td>Slowly perfused tissue</td>
<td>0.5396*</td>
</tr>
<tr>
<td>Blood</td>
<td>0.059*</td>
</tr>
<tr>
<td>Partition coefficients</td>
<td></td>
</tr>
<tr>
<td>Liver:blood</td>
<td>8.9*</td>
</tr>
<tr>
<td>Fat:blood</td>
<td>490*</td>
</tr>
<tr>
<td>Slowly perfused tissue: blood</td>
<td>3*</td>
</tr>
<tr>
<td>Rapidly perfused tissue: blood</td>
<td>8.4*</td>
</tr>
</tbody>
</table>

*The numbers in parentheses are the values during exercise periods. During exercise, blood flow rates to the rapidly perfused tissue and liver remained the same, while blood flow rates to the fat and slowly perfused compartments increased, with 21 and 79% of the increase in blood flow going to the fat and slowly perfused tissues, respectively. The increase in blood flow rate to fat was based on Bulow and Madsen (1978).

*From Brown et al. (1997). The sum of the fraction of body weight in each compartment is 0.91 because 9% of the body was assumed to receive minimal blood flow. The specific gravity of all tissues was assumed to be 1.

*The arterial and venous blood compartments were estimated to contain 35 and 65% of the blood volume, respectively.

*Calculated using blood:air and tissue:air partition coefficients measured in vitro in the rat from Andersen et al. (2001).

*This parameter was set to 3 for reasons discussed in Materials and Methods.

![Figure 4](https://example.com/fig4.png)

**FIG. 4.** Schematic diagram of the D₄ metabolism submodel.
These parameters were estimated by comparing model output to the cumulative amount of chemical absorbed during the exposure, the concentration of D₄ in the exhaled breath after the exposure, the concentration of D₄ in the plasma, and the concentrations of total metabolites in the plasma and urine. Although the concentrations of D₄ and metabolites were determined in both the whole blood and the blood plasma, parameter estimation was done using blood plasma concentrations. The concentrations of individual metabolites in the urine were also used to estimate parameters for the metabolism model.

Model equations were solved using two different software packages: Berkeley Madonna™ and ACSL™ (Aegis Technologies). Optimum parameters were found using the multiple curve-fitting routine in Berkeley Madonna, which minimizes the root mean square deviation between the data points and the model output. When multiple data sets were used for parameter estimation (e.g., time course data for the amount of several different metabolites in urine), the residuals from each dataset were weighted by the inverse of the SD of the dataset since the datasets were assumed to have a constant coefficient of variance. For the refined model, after completing a global estimation of the best-fit parameters, we independently adjusted Kmtp and CLmtp to improve the fit for blood plasma concentrations taken at 1500 h and adjusted KDF to provide a better fit for exhaled breath concentrations for times longer than 1500 h. Because the concentrations in blood and exhaled breath at the longest time were more than four orders of magnitude smaller than those during exposure, the curve-fitting algorithm was relatively insensitive to these latter data points. When estimating multiple parameters by curve fitting, increasing the number of parameters to be fit increases the risk that the program will find a local minimum of residuals instead of the global minimum. To prevent identifying a combination of parameters resulting in a local optimum instead of a global optimum, the optimizer was run using several different starting values (i.e., initial guesses of parameter values). Additionally, by limiting the values of parameters to physically plausible values, unrealistic solutions were prevented and computational time was minimized (MGA Software, 1997).

Sensitivity analysis. Identification of the key parameters affecting a pharmacokinetic measurement is important for calculating multiple parameters by curve-fitting with multiple datasets (Andersen et al., 2001). For example, a key parameter for determining the D₄ concentration in exhaled air and blood plasma during the exposure and washout period is Pb. By fitting this parameter independently to data that are sensitive to the parameter (i.e., holding other parameters constant), its value can be constrained for the multiple curve-fitting algorithm, reducing the computational time required by the computer and the risk of calculating an unrealistic value of the parameter based on datasets lacking sensitivity to the parameter.

To determine sensitivity of the model output to the parameters estimated using the experimental data, log-normalized sensitivity parameters (LSPs), defined in Clewell et al. (1994) as

\[ \text{LSP} = \frac{\partial \ln R}{\partial \ln x} \]  

were calculated. In Equation 1, R is the model output (i.e., for the calculations performed here, the concentrations of D₄ in the venous blood plasma and exhaled breath) and x is the parameter for which sensitivity is being determined. By this definition, the percentage change in model output due to a percentage change in a parameter is quantified, and thus the LSP value represents the relative importance of a parameter to model output. Values of LSP were calculated using the average values of the inhaled D₄ concentration and physiological properties (Table 1) and the cumulative amount of metabolite in the urine. Although model calculations are presented for average parameter values, parameter values for each individual subject are reported to illustrate the interindividual variability in parameter values. As expected, the refined model more accurately described the pharmacokinetic profiles. Although the model prediction of the D₄ concentration in exhaled breath is high during the first 500 min postexposure, in general the refined model describes the concentration of D₄ in exhaled breath and blood plasma, the concentration of total metabolite in blood plasma, and the cumulative amount of metabolite in the urine.

After the exposure ended, the D₄ concentration in exhaled breath, Cex, decreased by more than four orders of magnitude.

RESULTS

General Trends

Unlike many lipophilic chemicals with long half-lives for elimination from the body, elimination of D₄ (logKw/w = 5.1) from blood was rapid. After humans were exposed to 10 ppm [¹⁴C]-D₄ by inhalation, D₄ blood concentrations decreased rapidly due to exhalation and metabolism (Table 3). In the 30-min period following the exposure, about 13% of the absorbed dose of D₄ was eliminated by exhalation. Metabolites were detected in the blood at the earliest sampling time (30 min after the exposure began), and the first urine sample taken 30 min after the exposure had ended also had detectable levels of metabolites.

Model Structure

Consistent with the pharmacokinetics of D₄ in the rat, the refined model more accurately described the disposition of D₄ in the plasma and exhaled air (Fig. 5). For example, both the simple and refined model could match the concentration of D₄ in exhaled air, but only the refined model could simultaneously match the concentration of D₄ in blood plasma and exhaled air. Simulated D₄ exhaled breath concentrations increased between 10 to 20 min and 40 to 50 min (i.e., during the exercise periods) due to the physiological changes from exercise that were incorporated in the model. This increase in exhaled D₄ concentrations is consistent with the pharmacokinetic study results of Utell et al. (1998), who noted decreased retention efficiency of D₄ during the exercise periods.

Average values of D₄ concentrations in exhaled breath and plasma, total metabolite concentration in the plasma, and cumulative amount of metabolite in the urine for the six subjects exposed to [¹⁴C]-D₄ are shown in Figure 6. The simulated curves were calculated using the average values of the inhaled D₄ concentration and physiological properties (Table 1) and the average values of calculated model parameters (Table 4). Although model calculations are presented for average parameter values, parameter values for each individual subject are reported to illustrate the interindividual variability in parameter values. As expected, the refined model more accurately described the pharmacokinetic profiles. Although the model prediction of the D₄ concentration in exhaled breath is high during the first 500 min postexposure, in general the refined model describes the concentration of D₄ in exhaled breath and blood plasma, the concentration of total metabolite in blood plasma, and the cumulative amount of metabolite in the urine.
During the exposure, the prime determinant of plasma concentration was Pb (Table 5). At the later times, the return of D₄ to plasma from the lipophilic storage tissues in the body was also a prime determinant of the concentration of D₄ in exhaled air. While the overall time course for Cₑₓ, with the precipitous decline over time, was accounted for by the simple model (Fig. 6), the curve decreased too rapidly and leveled off at a concentration higher than the data. With this simple model, the characteristics of the slowly perfused compartment, representing muscle and skin, determined the curvature between 100 and 1500 min.

Describing the exhalation time course data more accurately

**TABLE 3**

Summary of D₄ Absorption, Metabolism, and Excretion

<table>
<thead>
<tr>
<th>Measured quantity</th>
<th>Time of estimation</th>
<th>Data*</th>
<th>Simple model</th>
<th>Refined model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount that entered the lungs (mg)</td>
<td>End of exposure (60 min)</td>
<td>154 ± 39</td>
<td>158 ± 38</td>
<td>158 ± 38</td>
</tr>
<tr>
<td>Amount retained (mg)</td>
<td>End of exposure (60 min)</td>
<td>19 ± 6</td>
<td>17 ± 3</td>
<td>14 ± 2</td>
</tr>
<tr>
<td>Concentration of D₄ in plasma (µg/l)</td>
<td>End of exposure (60 min)</td>
<td>115 ± 50</td>
<td>60 ± 14</td>
<td>121 ± 39</td>
</tr>
<tr>
<td>Concentration of metabolites in plasma (µg D₄ eq./l)</td>
<td>1 day postexposure</td>
<td>3.1 ± 2.9</td>
<td>0.028 ± 0.014</td>
<td>1.8 ± 1.3</td>
</tr>
<tr>
<td>Cumulative amount of D₄ exhaled after exposure ended (mg)</td>
<td>30-min period following the exposure</td>
<td>2.5 ± 0.6</td>
<td>3.1 ± 1.2</td>
<td>2.7 ± 1.1</td>
</tr>
<tr>
<td>Cumulative amount of D₄ metabolites excreted in urine (mg D₄ eq.)</td>
<td>1 day postexposure</td>
<td>2.2 ± 0.3</td>
<td>2.0 ± 0.4</td>
<td>2.1 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>8 days following the exposure</td>
<td>ND</td>
<td>9.9 ± 2.1</td>
<td>5.7 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>8 days following the exposure</td>
<td>5.0 ± 0.9c</td>
<td>5.3 ± 1.7c</td>
<td>4.6 ± 0.9c</td>
</tr>
</tbody>
</table>

*Average values ± one SD for n = 6.
ND, not determined.
Data were only available for Subjects 4 through 6, and so the average simulated value only included Subjects 4 through 6 for a fair comparison.

**FIG. 5.** Measured and calculated D₄ concentrations in exhaled breath and blood plasma as a function of time for subjects (A) 1, (B) 2, (C) 3, (D) 4, (E) 5, and (F) 6 of the primary study. Theoretical curves were calculated with the simple (dashed curve) and refined (solid curve) models.
required the addition of diffusion limitations in storage for both the slowly perfused and the fat compartments of the refined model. Several characteristics of the refined model dominated the behavior in different time periods. The characteristics of the slowly perfused compartment were critical in fitting the transitional period until about 1000 min and required incorporation of a mass-transfer limitation on uptake and release of chemical from this compartment. After about 2000 min, the more stable blood level of D₄ was determined by return from the fat compartment. If a flow-limited fat compartment without a deep compartment were included, the model overestimated the exhaled concentrations at longer times as shown by the dotted line. The presence of a diffusion-limited fat compartment allowed adjustment of the model fit for the final period of exhalation after 3000 min. The exhalation behavior of D₄ is a sensitive monitor of the characteristics of lipid storage compartments because of the rapid clearance of D₄ by exhalation and metabolism after its release from storage sites. Further refinements in the fit to the exhaled breath curve were possible by subdividing the slowly perfused compartment (e.g., as in Jonsson et al., 2001). We did not believe that an increase in model complexity was warranted by the limited data available from the single exhaled breath data set.

**Model Validation**

The refined model was then applied to a second data set (Utell et al., 1998). Using average characteristics and exposure conditions of the eight male and four female subjects as input for the model (Table 1), concentrations of D₄ in exhaled breath and blood plasma were simulated for male and female subjects.

![FIG. 6](https://example.com/fig6.png)

**FIG. 6.** Semilog plots of the average concentration of D₄ in (A) exhaled breath and (B) blood plasma and plots of (C) the concentration of total metabolite in blood plasma and (D) the average cumulative amount of total metabolite in urine as a function of time. The error bars represent one SD for n = 5 or 6. The simulated curves were calculated using the refined model (solid curves) and simple model (dashed curves) with average best-fit parameters, physiological properties, and exposure conditions.

---

**TABLE 4**

Parameters Calculated by Fitting the Model to the Data Obtained following Inhalation Exposures of Six Male Subjects to ¹⁴C-D₄

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Subject</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Average ± one SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple model</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pb</td>
<td>0.72</td>
<td>0.89</td>
<td>1.0</td>
<td>0.63</td>
<td>1.02</td>
<td>0.69</td>
</tr>
<tr>
<td>KFC (l/min/kg⁰.⁷⁺)ᵇ</td>
<td>0.071</td>
<td>0.065</td>
<td>0.047</td>
<td>0.090</td>
<td>0.2⁺</td>
<td>0.2⁺</td>
</tr>
<tr>
<td>KEL (l/min)</td>
<td>0.055</td>
<td>0.038</td>
<td>0.045</td>
<td>0.039</td>
<td>0.020</td>
<td>0.026</td>
</tr>
<tr>
<td>VDISC (l/kg)</td>
<td>0.87</td>
<td>1.82</td>
<td>1.2</td>
<td>0.98</td>
<td>1.6</td>
<td>0.82</td>
</tr>
<tr>
<td>Refined model</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pb</td>
<td>0.92</td>
<td>1.0</td>
<td>1.3</td>
<td>0.75</td>
<td>0.86</td>
<td>0.90</td>
</tr>
<tr>
<td>KFC (l/min/kg⁰.⁷⁺)ᵇ</td>
<td>0.052</td>
<td>0.11</td>
<td>0.036</td>
<td>0.065</td>
<td>0.2⁺</td>
<td>0.12</td>
</tr>
<tr>
<td>KEL (l/min)</td>
<td>0.057</td>
<td>0.033</td>
<td>0.041</td>
<td>0.036</td>
<td>0.033</td>
<td>0.029</td>
</tr>
<tr>
<td>VDISC (l/kg)</td>
<td>1.0</td>
<td>2.5</td>
<td>1.1</td>
<td>0.88</td>
<td>1.1</td>
<td>0.70</td>
</tr>
<tr>
<td>Kmlp (l/min)</td>
<td>0.020</td>
<td>0.060</td>
<td>0.022</td>
<td>0.022</td>
<td>0.044</td>
<td>0.15</td>
</tr>
<tr>
<td>CLmlp (l/min)</td>
<td>0.010</td>
<td>0.018</td>
<td>0.010</td>
<td>0.0080</td>
<td>0.010</td>
<td>0.025</td>
</tr>
<tr>
<td>KFD (l/min)</td>
<td>0.0043</td>
<td>0.0028</td>
<td>0.0019</td>
<td>0.0019</td>
<td>0.0078</td>
<td>0.0043</td>
</tr>
<tr>
<td>KDF (l/min)</td>
<td>0.0015</td>
<td>0.002</td>
<td>0.001</td>
<td>0.0012</td>
<td>0.0019</td>
<td>0.0050</td>
</tr>
<tr>
<td>PAS (l/min)</td>
<td>0.30</td>
<td>0.61</td>
<td>0.30</td>
<td>0.33</td>
<td>0.43</td>
<td>0.21</td>
</tr>
</tbody>
</table>

*Average for six subjects ± one SD.

ᵇMetabolic clearance in the liver, KF, is calculated as KFC × BW⁰.⁷.

The volume of distribution for the combined metabolite pool, VDISC, is calculated as VDISC × BW.

⁺⁺KFC was high enough that the rate of metabolism was controlled by blood flow to the liver and KFC could not be calculated from the data for this subject. The value of KFC was set so that the average value of KFC resulted in the model matching the average cumulative amount of metabolite in the urine eight days after the exposure had ended.
using the average parameter values of the refined PBPK model (Table 4). The prediction (Fig. 7) agrees very well with these earlier results. However, since this dataset is similar to the data used for model construction, it might be better to regard this as a test of reproducibility rather than a strict validation of the model. Although the model parameters were estimated using data from a study of six male subjects, the model was able to predict exposure outcomes for female subjects of the previous study.

Metabolism Model

Consistent with metabolism studies performed in the rat (Varaprath et al., 1999), methylsilanetriol (monomer-triol) and dimethylsilanediol (monomer-diol) were the major metabolites in humans (Fig. 8). Other metabolites in urine common to both rat and human metabolism of D₄ were tetramethyldisiloxane-1,3-diol (dimer-diol), dimethyldisiloxane-1,3,3,3-tetrol (dimer-tetrol), and hexamethyltrisiloxane-1,5-diol (trimer-diol; Table 6). Additionally, trimethyldisiloxane-1,3,3-triol (dimer-triol) was a very minor metabolite in human urine. Although the exact mechanism of D₄ metabolism is unknown, these metabolites can be incorporated into a proposed scheme for metabolism (Fig. 3) and assigned compartments in a metabolism model (Fig. 4). In all the proposed pathways, complete hydrolysis leads to 1 mol of monomer-triol and 3 mol of monomer-diol. However, intermediates may also be filtered into urine. Large quantities of monomer-triol were detected in the urine soon after the exposure ended, indicating that the reaction of tetramer-triol to form monomer-triol and trimer-diol is likely to be the major pathway for metabolism.

One potential problem with the mechanism shown in Figure 3 is that dimer-tetrol does not appear in the mechanism. The formation of dimer-tetrol could be explained by another minor pathway. During the initial step of the oxidation of D₄, if both methyl groups on an Si were oxidized, silicic acid, i.e., Si(OH)₄, could be formed. In the urine, which has a higher concentration of metabolites than the blood, a condensation reaction between silicic acid and monomer-diol could produce dimer-tetrol.

During the primary study, ¹⁴C-CO₂ was detected in exhaled breath in concentrations that decreased exponentially and reached very low levels by 24 h after the exposure ended. It is likely that ¹⁴C-CO₂ was produced from the oxidation of the methyl group from the intact D₄ molecule (i.e., the first step in the metabolic pathway). Eight days after the exposure ended, about 26% of the D₄ had been eliminated by metabolism. Because only one of the methyl groups on the D₄ molecule is radiolabeled, about 3.25% (= 26%/8) of the radioactivity should be eliminated in the form of ¹⁴C-CO₂, but only about 1.5% of the uptake of radioactivity was recovered as ¹⁴C-CO₂. The exhaled breath was not analyzed for ¹⁴C-CO₂ during the exposure and the 30-min postexposure period. Thus, more than half of the ¹⁴C-CO₂ from the initial oxidation of D₄ could have

<table>
<thead>
<tr>
<th></th>
<th>In blood plasma</th>
<th>In exhaled air</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 min</td>
<td>2 h</td>
</tr>
<tr>
<td>PB</td>
<td>0.84</td>
<td>0.95</td>
</tr>
<tr>
<td>KFC (l/min/kg⁰.⁷)</td>
<td>-0.17</td>
<td>-0.59</td>
</tr>
<tr>
<td>Kmlp (1/min)</td>
<td>0.23</td>
<td>0.91</td>
</tr>
<tr>
<td>CLnlp (l/min)</td>
<td>-0</td>
<td>-0</td>
</tr>
<tr>
<td>KFD (1/min)</td>
<td>-0</td>
<td>-0</td>
</tr>
<tr>
<td>KDF (1/min)</td>
<td>-0</td>
<td>-0</td>
</tr>
<tr>
<td>PAS (l/min)</td>
<td>-0.075</td>
<td>0.026</td>
</tr>
</tbody>
</table>

Note. The sensitivity analysis was not performed for parameters KEL and VDISC because the calculated values of concentrations of D₄ in the blood plasma and exhaled breath are not affected by these parameters. Values of LSP between -0.01 and 0.01 were reported as ~ 0.
been eliminated without detection. Because less than 5% of the total radioactivity will be metabolized to $^{14}$C-CO$_2$, this loss was not expected to significantly affect modeling results, and was not included in the model development.

The submodel for metabolism of D$_4$ in humans (Fig. 4), developed to be consistent with the major pathway of the proposed reaction mechanism (Fig. 3), was then combined with the refined PBPK model. The equations are provided in Appendix 1. The metabolism submodel required values for seven additional parameters: the allometric scaling constants for the first-order metabolism rate constants of tetramer-triol, trimer-diol, and dimer-diol (i.e., K1C, K2C, and K3C, respectively) and the first-order rate constants for the elimination of monomer-triol, monomer-diol, dimer-diol, and trimer-diol into urine (i.e., KEL1, KEL2, KEL3, and KEL4, respectively). These seven parameters were determined by minimizing the difference between model output and six datasets (i.e., the cumulative amount of monomer-triol, monomer-diol, dimer-diol, and trimer-diol in urine, the cumulative amount of total metabolite in urine, and the concentration of total metabolites in plasma). Parameter estimation results are listed in Table 7.

This metabolism submodel adequately described time course concentrations of three of the four individual metabolites in urine (i.e., monomer-diol, dimer-diol, and trimer-diol; Fig. 9). However, the model underpredicted the cumulative amount of monomer-triol in the urine at later times by up to 56%. It appears that further oxidative demethylation must occur after formation of the linear siloxanes to account for the net amounts of monomer-triol found in urine. The model structure was enlarged to include conversion of monomer-diol to monomer-triol (i.e., the model shown in Figure 4 was modified to include an additional reaction with rate constant K4). The expanded model was able to describe all the metabolite data (Fig. 9).

**DISCUSSION**

*Discovering the Requirement for the Refined Model*

For volatile organic compounds, the ratio of the chemical concentration in arterial blood, Ca, to the concentration in end-alveolar air, Calv, is expected to remain constant (i.e., $\frac{Ca}{Calv} = Pb$). In the D$_4$ study, blood samples were obtained from the venous blood, and a similar time-independent relationship is expected after the exposure ends. The equation often used to calculate Ca in PBPK models (e.g., the approach of Ramsey and Andersen, 1984, for inhalation exposures to styrene) is

$$Ca = \frac{QP \times Cin + QC \times Cv}{QC + QP/Pb} \tag{2}$$

where Cin is the concentration of chemical in the air entering the lungs and Cv is the concentration of chemical in the venous return. Equation 2 was developed by assuming that the absorbed chemical rapidly equilibrates between lung blood and lung air. Because Pb = Ca/Calv and after an exposure ends, Cin = 0, the following relationship can be derived:
After the exposure, the ratios \( \frac{C_v}{C_{alv}} \) and also \( \frac{C_v}{C_{ex}} \) should have remained constant, but did not (Fig. 10). The ratio \( \frac{C_v}{C_{ex}} \) increased substantially with time from about 5 soon after the exposure ended to over 400 one day after the exposure ended. This increase in \( \frac{C_v}{C_{ex}} \) occurred over a time period where the blood concentrations decreased by two orders of magnitude while the exhaled air concentrations decreased by more than four orders of magnitude.

The reason that blood concentrations did not fall as rapidly as the exhaled air concentrations was the presence of a pool of \( D_4 \) in blood that did not equilibrate with exhaled air, i.e., a pool of bound or sequestered \( D_4 \) that was unavailable for alveolar equilibration. Free \( D_4 \) was rapidly exhaled or metabolized; the bound \( D_4 \) was persistent in blood. The time evolution of the amount in this nonexchangeable blood compartment determined the values of \( K_{mlp} \) and \( C_{L_{mlp}} \). According to model calculations, when the exposure ended the fraction of \( D_4 \) in the blood that was unavailable was 0.24, but by 1 h following the exposure the fraction of unavailable \( D_4 \) had increased to 0.95.

Consistent with the results of Utell \textit{et al.} (1998), calculations with the refined model show that the half-life for \( D_4 \) clearance from the blood was shorter at early times than late times because the fraction of available \( D_4 \) in the blood decreased with time. When the exposure ended and the majority of \( D_4 \) in the blood was free \( D_4 \), the half-life for clearance of \( D_4 \) from the blood was about 1.4 h. However, later in the exposure when the majority of the \( D_4 \) in the blood was unavailable, the half-life for clearance was about 4.2 h. Due to the high sensitivity of blood concentrations at long times to \( C_{L_{mlp}} \) (Table 5), additional studies characterizing the nature of the pools of unavailable \( D_4 \) in blood are important.

The model structure for \( D_4 \) inhalation exposures in humans is perhaps more complex than warranted by the data because no tissue time-course concentration data were available. However, the model structure is also based on our experience in modeling \( D_4 \) pharmacokinetics in the rat. The premise in developing the PBPK model with \( D_4 \) in the rat was the assumption that the exhaled air \( D_4 \) was in equilibrium with a pool of free \( D_4 \) in blood. With this human model we retained the model structure required in the rat (i.e., the mobile-lipid pool

### TABLE 6

#### D₄ Metabolites Identified in Human Urine

<table>
<thead>
<tr>
<th>Label</th>
<th>Chemical name</th>
<th>Chemical formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trimer-diol</td>
<td>Hexamethyltrisiloxane-1,5-diol</td>
<td>Me₂Si(OH)-O-SiMe₂-O-Si(OH)Me₂</td>
</tr>
<tr>
<td>Dimer-diol</td>
<td>Tetramethyldisiloxane-1,3-diol</td>
<td>Me₂Si(OH)-O-Si(OH)Me₂</td>
</tr>
<tr>
<td>Dimer-triol</td>
<td>Trimethyldisiloxane-1,3,3-triol</td>
<td>Me₂Si(OH)-O-Si(OH)Me</td>
</tr>
<tr>
<td>Dimer-tetrol</td>
<td>Dimethyltrisiloxane-1,3,3-tetrol</td>
<td>Me₃Si(OH)₂</td>
</tr>
<tr>
<td>Monomer-diol</td>
<td>Dimethyldisiloxane</td>
<td></td>
</tr>
<tr>
<td>Monomer-triol</td>
<td>Methylsilanetriol</td>
<td></td>
</tr>
</tbody>
</table>

### TABLE 7

#### Metabolism Model Parameter Estimation Results

<table>
<thead>
<tr>
<th>Parameters</th>
<th>When ( K₄ = 0 )</th>
<th>When ( K₄ ≠ 0 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( K_{1C} ) (l/min/kg(^{0.7}))</td>
<td>0.14</td>
<td>&gt; 0.3</td>
</tr>
<tr>
<td>( K_{2C} ) (l/min/kg(^{0.7}))</td>
<td>0.063</td>
<td>0.035</td>
</tr>
<tr>
<td>( K_{3C} ) (l/min/kg(^{0.7}))</td>
<td>0.011</td>
<td>0.017</td>
</tr>
<tr>
<td>( K_{4C} ) (l/min/kg(^{0.7}))</td>
<td>—</td>
<td>0.0045</td>
</tr>
<tr>
<td>( K_{EL1} ) (l/min)</td>
<td>&gt; 0.3(^*)</td>
<td>&gt; 0.3(^*)</td>
</tr>
<tr>
<td>( K_{EL2} ) (l/min)</td>
<td>0.022</td>
<td>0.040</td>
</tr>
<tr>
<td>( K_{EL3} ) (l/min)</td>
<td>0.027</td>
<td>0.040</td>
</tr>
<tr>
<td>( K_{EL4} ) (l/min)</td>
<td>0.083</td>
<td>0.046</td>
</tr>
</tbody>
</table>

\(^*\) This parameter could not be calculated because the rate of mass transfer of monomer-triol into the urine was controlled by the rate of production of monomer-triol.

Note. Parameters were calculated both with and without an extra demethylation step (see Fig. 4).

\( \frac{C_v}{C_{alv}} = P_b + \frac{Q_P}{Q_C}. \) (3)
concept with production from the liver, movement through the plasma, and clearance to the fat). Further research will be necessary to identify the plasma lipids that sequester D₄.

Clearance of Circulating D₄

Although D₄ has a large fat:blood partition coefficient, it is not expected to undergo extensive bioaccumulation due to rapid clearance from the body by exhalation and metabolism. With xenobiotic compounds, elimination processes are frequently described by clearances (i.e., the volume of blood from which chemical is lost per time, with units of flow). With D₄, we can calculate clearances by the two major pathways of elimination, exhalation and hepatic clearance. Based on the average values of KFC (Table 4) and liver blood flow (Table 2), the average liver clearance was 0.85 l/min compared to a liver blood flow of 1.29 l/min. At the extreme, with KFC = 0.2 l/min/kgᵦ², hepatic clearance was 1.01 l/min (i.e., nearly 80% of hepatic blood flow). Under these conditions, hepatic clearance is essentially limited by blood flow to the liver. Clearance by exhalation, CLEX, can also be calculated from model parameters. In the postexposure period, CLEX is calculated as

\[
\text{CLEX} = \text{QC} \times \left( \frac{\text{Cv} - \text{Cu}}{\text{Cv}} \right). \tag{4}
\]

Combining this relationship with Equation 2 for Cin = 0 gives

\[
\text{CLEX} = \frac{\text{QP}}{\text{Pb} + \text{QP/QC}}. \tag{5}
\]

For compounds like D₄ with small values of Pb, CLEX is high. For a Pb of 1.0 and resting QC and QP values of 5.03 and 7.83 l/min, CLEX was 3.06 l/min. Total clearance from hepatic metabolism and exhalation was 3.91 (0.85 + 3.06) l/min (i.e., about 60% of QC).

Metabolic clearance of D₄ simply accounts for those reactions that metabolize D₄ to downstream products. Because of the ability to speciate metabolites in urine over time, it was also possible to infer the metabolic pathway for the group of linear siloxanes produced from oxidation and ring cleavage. The sequential model with rapid production of the trimer-diol after oxidation followed by hydrolysis is sufficient to describe the results as long as a second oxidative pathway is provided for conversion of monomer-diol to monomer-triol. This latter step may be more favored than oxidation of other intermediates due to longer persistence of the monomer-diol than any of the other longer chain metabolites that serve as relatively shorter-lived intermediates. This model infers time course concentrations in the blood from the appearance of metabolites in urine. The relationships of blood and urinary concentrations depend on biotransformation and elimination rates.

In rodents, inhalation of D₄ produces a dose-related hepatomegaly, transient hepatic hyperplasia, hypertrophy, and induction of cytochrome P450 enzymes in a manner similar to phenobarbital (McKim et al., 1998, 2001). This pattern of induction was also observed following oral exposure to D₄ (Zhang et al., 2000). Although several hepatic enzymes are affected by D₄ exposure, CYP2B1/2 enzymes are induced to the greatest extent. In addition, it appears that these enzymes are also capable of recognizing D₄ as a substrate and therefore may play an important role in its elimination (Salyers et al., 1996). While there may eventually be value in a model for the metabolites to assess total exposures to siloxanes, development of a pharmacokinetic/pharmacodynamic model to study concentration response data for protein induction, enzyme activity, and liver weight changes demonstrated that the hepatic responses following D₄ exposure are more likely to be related to parent D₄ than to metabolites (Sarangapani et al., 2002). Additional evidence for the role of parent D₄ is from structural reasons in relation to other xenobiotics that have phenobarbital-like induction in liver (Waxman, 1999). Unfortunately there is no specific information available on the toxicity of the individual metabolites for comparison with the studies of parent D₄.

Blood:Air Partition Coefficient

The average value of Pb for humans estimated by fitting the model to the in vivo data was 0.96. The in vitro partition coefficient between rat blood and air was determined to be 4.3, while the Pb value required to describe the rat inhalation kinetics in vivo was also close to 1.0 (Andersen et al., 2001). Several investigators have noted that Pb values for volatile compounds are usually larger by about a factor of two for rat blood compared to human blood (Gargas et al., 1989). Thus, all available results are consistent with a Pb for D₄ in humans near unity.
Recently, Luu and Hutter (2001) developed a PBPK model for D₄ inhalation exposures in humans and rats that used a much different Pb, i.e., 20. The reasons for their use of a much larger Pb value were due to an oversight in the development of their rat model and the application of an unnatural constraint in the development of their human model (Andersen et al., 2002). They used total radioactivity in blood from the rat inhalation study without differentiating parent D₄ from metabolites. Thus, their model fit the combined amount of D₄ and metabolites in blood as if it were parent D₄, even though by the end of the 6-h inhalation exposure in rats, the majority of radioactivity in blood was metabolite. Because of this oversight, a higher Pb value was required to maintain higher blood concentrations over time than due to D₄ alone. In their human model, they applied the higher Pb to the human data set of Utell et al. (1998). They then used the total amount absorbed during exposure, about 11% of the total inhaled, not as an outcome to be matched by the model, but as a physical constraint. With this unnatural constraint for a PBPK model with a volatile compound, a much higher Pb is required to retain all inhaled compound. Our PBPK model for D₄ was developed following the common approach for inhalation exposures to volatile compounds and describing D₄ plasma concentrations without artificial constraints on uptake. Because our model described Ca using Equation 2, the proportion retained was an output of the model and not a constraint.

Retention Efficiency

Recently, Csanady and Filser (2001) modeled the effects of Pb, QC, and QP on chemical uptake during inhalation exposures with varying workloads, noting that increased physical activity can increase uptake. The effect of exercise on absorption during inhalation exposures can be studied using Equation 2. For a short time early in an exposure when the concentration in the venous return is low (i.e., CV = 0), the proportion of absorbed chemical is as large as possible, and Equation 2 becomes

$$\frac{Ca}{Cin} = \frac{QP/QC}{1 + (QP/QC)/Pb}.$$  (6)

For chemicals with a high Pb, Ca/Cin would be approximately equal to QP/QC. The ratio QP/QC increases with exercise. For example, the ratio was experimentally determined to be about 0.8 at rest and about 2.0 at a workload of 50 W by Astrand (1983). Thus, exercise would increase the amount of chemical absorbed into the blood stream. However, for chemicals like D₄ with a low Pb, Ca/Cin will not be greatly affected by increasing QP/QC. A plot of Ca/Cin as a function of Pb, as predicted by Equation 6, shows that early in an exposure the effect of exercise on the ratio of Ca/Cin is minor for chemicals with Pb < 1 (Fig. 11A). This result is consistent with Csanady and Filser, who concluded that for chemicals with a Pb > 6, physical activity at work should be taken into account when determining occupational limit concentrations in air.

Exercise can also affect the fraction absorbed, FA (i.e., the fraction of chemical entering the lungs that absorbs into lung blood), defined as

$$FA = \frac{Cin - Cex}{Cin}.$$  (7)

To determine Cex, Calv (i.e., Ca/Pb) must be adjusted for the concentration of chemical in the dead space of the lungs as follows:

$$Cex = FDS \times Cin + (1 - FDS) \times Calv.$$  (8)

where FDS is the fraction of dead space in the lungs, which also decreases with exercise. In the study of Astrand (1983), FDS was about 0.33 at rest and about 0.11 with a workload of 50 W. Equation 9, developed from Equations 6, 7, and 8, shows the effect of increasing QP/QC on FA:
in humans following inhalation exposures. D₄ 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, and rapid elimination from all tissues other than fat after cessation of exposure. This PBPK model for D₄ provides a tool to quantify transport of highly lipophilic chemicals throughout the blood and tissues and to quantitatively characterize the retention, distribution, and elimination of parent D₄ and its hydrolysis and oxidation products from the body following inhalation exposures to humans. The human D₄ model is similar to the rat model, with similar partition coefficients and compartmental structures. The success in developing consistent PBPK models for D₄ in two species indicates that this human model should be useful for simulating tissue dosimetry of D₄ in humans exposed by inhalation and to further support the risk assessment of D₄.

**APPENDIX 1**

**Simple Model**

By modeling the lungs as a well-mixed compartment with an average, one-directional airflow in the region of gas exchange (i.e., QP), and with rapid equilibration between lung air and blood in the lung alveoli, the concentration in the blood exiting the lungs, Cₐ, can be described as

\[
C_a = \frac{Q_P \times C_{in} + Q_C \times C_{BLV}}{Q_C + Q_P/P_d} \tag{A-1}
\]

where CBLV is the free concentration of D₄ in the venous blood compartment and Cin was 10 ppm D₄ during the exposure and zero after the exposure ended. The concentration of D₄ in exhaled breath, Cex, can be calculated using Equation 8, with Cal = Cₐ/Pd. Separate compartments were included for the venous and arterial blood. The mass balance for the concentration of D₄ in the arterial blood compartment, CBLA, is

\[
\frac{dC_{BLA}}{dt} = QC \times (C_a - C_{BLA}) \tag{A-2}
\]

where t is time and VBLA is the volume of the arterial blood compartment. The rate of mass transfer of D₄ into the fat, rapidly and slowly perfused tissues, and liver was limited by the blood flow to the tissues, as shown in A-3 to A-6:

\[
\frac{dC_{F}}{dt} = \frac{Q_F \times (C_{BLA} - C_{F})}{P_F} \tag{A-3}
\]

\[
\frac{dC_{R}}{dt} = \frac{Q_R \times (C_{BLA} - C_{R})}{P_R} \tag{A-4}
\]

\[
\frac{dC_{S}}{dt} = \frac{Q_S \times (C_{BLA} - C_{S})}{P_S} \tag{A-5}
\]

\[
\frac{dC_{L}}{dt} = \frac{Q_L \times (C_{BLA} - C_{L})}{P_L} - K_F \times \frac{C_{L}}{P_L} \tag{A-6}
\]

where CF, CR, CS, and CL are the concentrations of D₄ in the fat, rapidly and slowly perfused tissues, and liver, respectively. Because D₄ is metabolized in the liver, A-6 includes the first-order clearance constant KF. The blood flows from the organ compartments combine in a mixed venous return compartment.

![Graph](https://academic.oup.com/toxsci/article-abstract/72/1/3/1648577/7166467)
where VBLV is the volume of the venous blood compartment. A one-compartment model was used to describe the disposition of the pooled metabolites with first-order renal clearance as shown in A-8:

$$\frac{dC_{BLV}}{dt} = K_F \times \frac{CL_{PL}}{PL} - K_{EL} \times C_{MET} \quad (A-8)$$

where CMET and VDIS are the blood plasma concentration and volume of distribution of the combined metabolite pool, respectively. The amount of total metabolite excreted into urine, AMEX, can be calculated using the equation

$$\frac{dAMEX}{dt} = K_{EL} \times C_{MET}. \quad (A-9)$$

At the beginning of the exposure, the concentration of unavailable D₄ in all compartments was assumed to be zero (i.e., CA = 0, CBLA = 0, CR = 0, VBLA = 0, and CBLV = 0 at t = 0), and the concentration of metabolite in the blood plasma and the amount of metabolite in the urine were assumed to be zero (i.e., CMET = 0 and AMEX = 0 at t = 0).

Refined Model

The refined model has a mobile lipid pool in blood with D₄ that does not equilibrate with free D₄ in blood. This lipid pool is generated in the liver and cleared by the fat. The mass balance on the liver, A-6, then becomes

$$\frac{dV_L}{dt} = Q_L \times \left( \frac{C_{BLA} - CL_{PL}}{PL} \right) - K_F \times \frac{CL_{PL}}{PL} \times K_{mlp} \times V_L \times \frac{CL_{PL}}{PL}. \quad (A-10)$$

This sequestered D₄ in blood is cleared in the fat compartment, where D₄ can also move into a deep fat compartment, and so Equation A-3 becomes

$$\frac{dV_F}{dt} = Q_F \times \left( \frac{C_{BLA} - CL_{PL}}{PL} \right) + Q_F \times \frac{CL_{mlp}}{PL} \times CA_{mlp} - K_{FD} \times CF \times V_F + K_{FD} \times AFD. \quad (A-11)$$

where CA_{mlp} is the concentration of unavailable D₄ (i.e., in the mobile lipid pool) in the arterial blood and AFD is the amount of D₄ in the deep fat compartment, which is described as

$$\frac{dAFD}{dt} = K_{FD} \times CF \times V_F - K_{FD} \times AFD. \quad (A-12)$$

The mass balances for unavailable D₄ in the arterial and venous blood are

$$\frac{dC_{Ampl}}{dt} = QC \times (C_{Vmlp} - CA_{mlp}) - \frac{Q_F \times CL_{mlp}}{Q_F + CL_{mlp}} \times CA_{mlp} \quad (A-13)$$

$$\frac{dC_{Vmlp}}{dt} = K_{mlp} \times V_L \times CL_{PL} - QC \times (C_{Vmlp} - CA_{mlp}) \quad (A-14)$$

where CV_{mlp} is the concentration of unavailable D₄ (i.e., in the mobile lipid pool) in the venous blood. The average concentration of total D₄ (i.e., free and bound) in the mixed venous return, CV_{mix}, can be calculated as

$$CV_{mix} = C_{BLV} + CV_{mlp}. \quad (A-15)$$

At the beginning of the exposure, the concentration of unavailable D₄ in both the arterial and venous blood is assumed to be zero (i.e., CA_{mlp} = 0 and CV_{mlp} = 0 at t = 0).

In the refined model, the mass transfer in the slowly perfused compartment was limited by diffusion into the tissue. The mass balance on the slowly perfused compartment becomes

$$\frac{dCS}{dt} = PAS \times \left( \frac{C_{BLA} - CS}{PS} \right). \quad (A-16)$$

In this case, the blood exiting the slowly perfused tissue compartment has not equilibrated with the compartment (i.e., the concentration in blood plasma leaving the slowly perfused compartment, CVS, is not equal to CS/PS), as illustrated by A-17:

$$CV_{S} = C_{BLA} - PS \times \left( \frac{C_{BLA} - CS}{PS} \right). \quad (A-17)$$

Finally, the mass balance equation for the venous return becomes

$$\frac{dV_{BLV}}{dt} = Q_F \times \frac{CF}{PF + QL \times CL_{PL} + QR \times CR}{PR} + Q_S \times CV_{S} - QC \times C_{BLV}. \quad (A-18)$$

For the refined model, A-18 replaces A-7.

A model describing the plasma concentration of metabolites tetramer-triol, trimer-diol, dimer-diol, and monomer-diol (i.e., CIVT, CIID, CIID, CID, and CIT, respectively) was also developed. Instead of including a one-compartment model for a combined metabolite pool, a one-compartment model was included for each individual metabolite. The volume of distribution for each metabolite was assumed to be the same as it was for the combined metabolite pool. The rate of change of the metabolite concentrations are described as follows:

$$\frac{dC_{IVT}}{dt} = K_F \times \frac{CL_{PL}}{PL} - K_1 \times C_{IVT} \quad (A-19)$$

$$\frac{dC_{IID}}{dt} = K_1 \times C_{IVT} - (K_2 + K_{EL4}) \times C_{IID} \quad (A-20)$$

$$\frac{dC_{IID}}{dt} = K_2 \times C_{IID} - (K_3 + K_{EL3}) \times C_{IID} \quad (A-21)$$

$$\frac{dC_{ID}}{dt} = K_2 \times C_{IID} + 2 \times K_3 \times C_{IID} - (K_4 + K_{EL2}) \times C_{ID} \quad (A-22)$$

$$\frac{dC_{IT}}{dt} = K_1 \times C_{IVT} + K_4 \times C_{ID} - K_{EL1} \times C_{IT} \quad (A-23)$$

where K₁, K₂, K₃, and K₄ are first-order reaction rate constants for the metabolism of tetramer-triol, trimer-diol, dimer-diol, and monomer-diol, respectively, as shown in Figures 3 and 4. The cumulative amount of monomer-triol, monomer-diol, dimer-diol, and trimer-diol in the urine (i.e., AIT, AID, AIID, and AIIID, respectively) can be calculated using the equations:
APPENDIX 2

Notation

AFD, amount of D₄ in the deep fat compartment
AID, amount of monomer-diol in the urine
AIID, amount of dimer-diol in the urine
AIIID, amount of trimer-diol in the urine
BW, body weight
Ca, concentration of D₄ in the arterial blood exiting the lungs
CALV, concentration of D₄ in alveolar air
CAMLp, concentration of unavailable D₄ in the arterial blood
CBLA, concentration of D₄ in the arterial blood compartment
CBLV, concentration of D₄ in the venous blood compartment
C’ex, concentration of D₄ in exhaled breath
CF, concentration of D₄ in the fat
CID, concentration of monomer-diol in the blood plasma
CIID, concentration of dimer-diol in the blood plasma
CIIID, concentration of trimer-diol in the blood plasma
Cin, inhaled concentration of D₄
CLC, concentration of D₄ in the liver
CLE, clearance of D₄ by exhalation
CMLP, clearance of unavailable D₄ from the blood into the fat
CMET, concentration of combined metabolite pool in blood plasma
CR, concentration of D₄ in the rapidly perfused tissue compartment
CS, concentration of D₄ in the slowly perfused tissue compartment
Cv, concentration of chemical in the venous return
CVmix, concentration of total D₄ (i.e., available and unavailable) in the venous return
CVS, concentration of D₄ in the plasma exiting the slowly perfused tissue compartment where there is a diffusional resistance to mass transport in that compartment
CVmlp, concentration of unavailable D₄ in the venous blood
FDS, fraction of the lung volume where gas exchange does not occur (i.e., dead space)
K₁, first-order reaction rate constant for the metabolism of tetramer-triol
K₁C, allometric constant for calculating K₁
K₂, first-order reaction rate constant for metabolism of trimer-diol
K₂C, allometric constant for calculating K₂
K₃, first-order reaction rate constant for the metabolism of dimer-diol
K₃C, allometric constant for calculating K₃
K₄, first-order reaction rate constant for oxidation of monomer-diol
K₄C, allometric constant for calculating K₄

\[
\frac{d\text{AIT}}{dt} = \text{KEL1} \times \text{CIT} \tag{A-24}
\]
\[
\frac{d\text{AID}}{dt} = \text{KEL2} \times \text{CID} \tag{A-25}
\]
\[
\frac{d\text{AIID}}{dt} = \text{KEL3} \times \text{CIID} \tag{A-26}
\]
\[
\frac{d\text{AIIID}}{dt} = \text{KEL4} \times \text{CIIID}. \tag{A-27}
\]

At the beginning of an exposure, the concentrations of all metabolites in blood plasma and the amount of all metabolites in the urine are assumed to be zero (i.e., \(\text{CIVT} = 0\), \(\text{CIID} = 0\), \(\text{CID} = 0\), \(\text{CIT} = 0\), \(\text{AIT} = 0\), \(\text{AID} = 0\), \(\text{AIID} = 0\), and \(\text{AIIID} = 0\) at \(t = 0\)).

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