Predicting Age-Appropriate Pharmacokinetics of Six Volatile Organic Compounds in the Rat Utilizing Physiologically Based Pharmacokinetic Modeling

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The capability of physiologically based pharmacokinetic models to incorporate age-appropriate physiological and chemical-specific parameters was utilized to predict changes in internal dosimetry for six volatile organic compounds (VOCs) across different ages of rats. Typical 6-h animal inhalation exposures to 50 and 500 ppm perchloroethylene, trichloroethylene, benzene, chloroform, methylene chloride, or methyl ethyl ketone (MEK) were simulated for postnatal day 10 (PND10), 2-month-old (adult), and 2-year-old (aged) rats. With the exception of MEK, predicted venous blood concentrations of VOCs in the aged rat were equal or up to 1.5-fold higher when compared to the adult rat at both exposure levels, whereas levels were predicted to be up to 3.8-fold higher in the case of PND10 at 50 ppm. Predicted blood levels of MEK were similar in the adult and aged rat, but were more than 5-fold and 30-fold greater for PND10 rats at 500 and 50 ppm, respectively, reflecting high water solubility along with lower metabolic capability and faster ventilation rate per unit body weight (BW) of PND10 animals. Steady-state blood levels of VOCs, simulated by modeling constant exposure, were predicted to be achieved in the order PND10 > adult > aged, largely due to increasing fat volume. The dose metric, total amount metabolized per unit liver volume was generally much lower in PND10 than in adult rats. The blood:air partition coefficient, fat volume, and fat blood flow were identified as critical determinants for the predicted differences in venous blood concentrations between the adult and aged. The lower metabolic capability, largely due to a smaller liver size, and faster ventilation rate per unit BW of PND10 animals contribute the most to the differences between PND10 and adult rats. This study highlights the pharmacokinetic differences and the relevant parameters that may contribute to differential susceptibility to the toxic effects of VOCs across life stages of the rat.

Key Words: volatile organic compounds (VOCs); physiologically based pharmacokinetic (PBPK) modeling; rat; age-dependent pharmacokinetics.

The pediatric and elderly populations require special considerations in risk assessment since they can exhibit increased or decreased sensitivity to the toxic actions of xenobiotics when compared to adults. The difference in sensitivity may be due to age-dependent changes in pharmacokinetics and/or pharmacodynamics. In contrast to pharmacodynamics which reflects the effect(s) of a chemical on the body, pharmacokinetics is defined by how the body handles the absorption, distribution, metabolism, and excretion of a particular chemical. These processes are dependent on physiological (e.g., ventilation rate and cardiac output [QC]) and chemical-specific (e.g., tissue:blood partition coefficients [PCs] and metabolism) parameters which undergo age-dependent changes. For instance, the immaturity of xenobiotic metabolizing enzyme systems in children has been identified as an important pharmacokinetic factor which can result in decreased overall clearance and higher internal doses (Clewell et al., 2004). For the same reason, children may also exhibit resistance to chemical toxicity if xenobiotic metabolizing enzymes are required for the bioactivation of a chemical to a toxic intermediate (Pineiro-Carrero and Pineiro, 2004). The combined effects of age-dependent pharmacokinetic changes can potentially yield significant differences in internal dosimetry across ages and several efforts have attempted to evaluate such effects in humans (Clewell et al., 2004; Ginsberg et al., 2004; Sarangapani et al., 2003).

Although much of the focus has been on humans, it is critical from a risk assessment perspective to also understand the age-dependent pharmacokinetic changes in experimental animals.
The frequently encountered lack of adequate human data for quantitative risk analysis has resulted in animal toxicity data being the predominant type of data used for establishing dose–response relationships and obtaining points of departure, such as a no-observed adverse effect level or lowest-observed adverse effect level. Furthermore, the ability to design well-controlled animal toxicity studies has allowed the application of a more mechanistic approach for risk assessment that emphasizes mode of action, i.e., the pharmacokinetic and pharmacodynamic processes that lead to the observed response (Andersen et al., 1987; Clewell et al., 2002).

Animal toxicity studies have also been useful for evaluating risks associated with early-life susceptibility to chemical toxicity. Studies involving in utero, lactational, and post-weaning exposures are now standard toxicity studies for evaluating developmental effects associated with chemical exposure. In contrast to approaches extrapolating from adults to younger ages, these studies offer the unique opportunity to identify critical window(s) of development during which the animal may be particularly susceptible to the toxic effects of a chemical. Risk analysis is improved when the equivalent developmental period can be identified in humans.

Although animal toxicity studies are extensively used for risk assessment, analyses for different life stages have been hampered by the lack of age-appropriate dosimetry. Because age-dependent differences in pharmacokinetics have largely been unaddressed, the question lingers whether the observed effects at different life stages are due to increased susceptibility from increased internal exposure for the same external exposure (pharmacokinetic differences), increased biological sensitivity (pharmacodynamic differences), or a combination of the two. Current dose–response analyses for developmental toxicity studies use solely the dose administered to the mother. Ideally, such analysis should be carried out with internal dosimetry corresponding to the specific age being examined for deleterious effects. In combination with information on the window of susceptibility, age-appropriate internal dosimetry could facilitate the extrapolation of risk to humans.

Rodent cancer bioassays are another type of animal toxicity studies for which age-appropriate pharmacokinetics have generally not been adequately considered. Such bioassays, which are designed to assess the carcinogenic potential of chemicals, begin after puberty and can last for up to 2 years (Barton et al., 2005). Age-related changes in physiological and chemical-specific parameters occurring during the time course of a rodent cancer bioassay could lead to significant changes in internal dosimetry which in turn could impact interpretation of results. To this end, information on age-dependent changes in internal dosimetry, particularly for the later ages, would be useful for optimizing the design of such assays and interpretation of their results.

The effects of age on the pharmacokinetics of xenobiotics can be evaluated using physiologically based pharmacokinetic (PBPK) models, which provide an anatomically/physiologically bounded quantitative structure within which to describe pharmacokinetic behavior. These models can be particularly useful in predicting tissue dosimetry from a given chemical exposure, and thus help reduce the uncertainties associated with dose–response analysis across dose, route, and species. PBPK modeling refines dose–response analysis, which has traditionally relied upon external exposures rather than internal doses of a chemical. The use of PBPK models in risk assessment is increasing and has been demonstrated for a number of chemicals including methylene chloride, styrene, trichloroethylene, vinyl chloride, and vinyl acetate (Krishnan and Johanson, 2005). Important to the study of age-dependent differences in pharmacokinetics is the capability of PBPK models to incorporate age-dependent changes in physiological and chemical-specific parameters to describe tissue dosimetry across life stages. Thus, PBPK models offer an approach for evaluating age-dependent pharmacokinetic differences in animal toxicity studies involving different life stages.

Volatile organic compounds (VOCs) are a group of chemicals widely used in industrialized nations. Their uses range from paint thinners, dry cleaning solvents, and constituents of petroleum fuel to intermediates used in the production of other chemicals. They are produced in high volume every year. For example, the production of perchloroethylene in the U.S. has been estimated to be on the order of 400 million pounds per year (Lash and Parker, 2001). An important chemical property of these compounds, intimately related to their toxicology, is their high vapor pressure which enables them to readily enter the atmosphere under normal conditions and become contaminants of air, soil, and water. Some of the toxicities associated with VOC exposure include developmental effects, cancer, and central nervous system (CNS) depression. Although their toxicology is relatively well-documented, age-dependent differences in susceptibility to their toxic effects remain poorly understood. Thus, in an effort to better understand the potential age-dependent changes in the internal dosimetry of VOCs that might arise in rats, age-appropriate physiological and chemical-specific parameters were assembled from the available literature and incorporated into a PBPK model. Six different VOCs, namely perchloroethylene, trichloroethylene, benzene, chloroform, methylene chloride, and methyl ethyl ketone (MEK), were analyzed across three different ages of the rat exposed by inhalation. The availability of experimentally determined age-appropriate PCs (Mahle et al., 2005, in press) was the basis for selecting this particular set of VOCs. PCs are critical parameters in PBPK modeling as they represent the equilibrium concentrations of a chemical achieved in air or tissue versus blood. Therefore, age-dependent differences in PCs, alone or in combination with other parameters, can lead to significant pharmacokinetic differences across life stages. Three ages were examined, namely postnatal day 10 (PND10), 2-month-old (adult), and 2-year-old (aged) rats. The results indicate that while the blood:air partition coefficient (PB) and parameters associated with fat tissue are
important in the pharmacokinetic differences between adult and aged, metabolism and ventilation rate per unit body weight (BW) appear to be the most critical when comparing the adult and PND10.

MATERIALS AND METHODS

Model Structure

The PBPK model used in this study is based on a model proposed to describe the pharmacokinetics of styrene (Ramsey and Andersen, 1984). The major modifications include separate compartments for kidney, gastrointestinal tract (GI), and brain as depicted in Figure 1. The model code was developed and run using acslXtreme (version 2.0.1.6, Aegis Technologies, Huntsville, AL).

Physiological Parameters

Adult and aged. Available age-appropriate data in the range of the adult and aged rat (Delp et al., 1998) indicated that BW-normalized cardiac output (referred to as cardiac index, QCI) was constant in contrast to the BW^{0.75} scaling convention suggested for comparisons across species. Thus, QCI values for a 187 and 438 g Fisher 344 rat (Delp et al., 1998) were taken from a report by Delp’s laboratory (Delp et al., 1998; Smith and Hutchins, 1979; Vizek and Albrecht, 1973). Alveolar ventilation rate (QP) was assumed to be equal to QC and scaled to BW to estimate the QC for a 200 (adult) and 450 g (aged) rat, respectively (Table 1). Tissue volumes and blood flows for the adult and aged rat were taken from a report by Delp’s laboratory (Delp et al., 1998) and scaled to BW. The rapidly perfused compartment for these two ages was made up of adrenal, heart, thyroid, lung, and salivary gland tissue, while skin, bone, skeletal muscle, reproductive, and other miscellaneous tissues made up the slowly perfused compartment.

Postnatal day 10. Due to lack of data in the early ages, QC for PND10 was estimated by plotting QC values versus BW as reported in the literature (Delp et al., 1998; Smith and Hutchins, 1979; Vizek and Albrecht, 1973) and analyzing by nonlinear regression for fit to the equation QC = QC_{max} \times BW / (BW_{50} + BW) (Graphpad Prism, San Diego, CA), where QC_{max} represents the maximum QC and BW_{50} the BW to reach 50% of this value. The best fitted values of QC_{max} and BW_{50} were 8.72 l/h and 0.189 kg, respectively. The goodness of the fit (r^2 = 0.82) prompted the estimation of QC for PND10 by these means (Table 1). QP was determined from minute ventilation data reported by Bandla et al. (1999) (Table 1) assuming a dead space volume of 0.82 l/h and 0.189 kg, respectively. The discrepancies between the sum of tissue masses and BW for the different ages likely reflect different experimental techniques and conditions used by different laboratories as well as unaccounted losses associated with processing animals such as loss of blood or the contents of the GI, urinary bladder, and seminal vesicles (Delp et al., 1998).

Chemical-Specific Parameters

Tissue:blood partition coefficients. Partition coefficients corresponding to PND10, adult 2-month-old, and aged (22 months old) Sprague–Dawley rats were taken from Mahle et al. (2005) and are listed in Table 2.
**TABLE 2**

<table>
<thead>
<tr>
<th>VOC</th>
<th>PB</th>
<th>PL (^b)</th>
<th>PK (^c)</th>
<th>PF (^d)</th>
<th>PBR (^e)</th>
<th>PR (^f)</th>
<th>PS (^g)</th>
<th>PC (^h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perchloroethylene</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PND10</td>
<td>15.0</td>
<td>2.81</td>
<td>2.15</td>
<td>63.08</td>
<td>1.69</td>
<td>2.81</td>
<td>6.19</td>
<td>6.19</td>
</tr>
<tr>
<td>Adult</td>
<td>13.6</td>
<td>2.57</td>
<td>2.40</td>
<td>112.43</td>
<td>2.97</td>
<td>2.57</td>
<td>1.84</td>
<td>1.84</td>
</tr>
<tr>
<td>Aged</td>
<td>20.9</td>
<td>3.15</td>
<td>1.80</td>
<td>95.79</td>
<td>2.79</td>
<td>3.15</td>
<td>2.89</td>
<td>2.89</td>
</tr>
<tr>
<td>Trichloroethylene</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PND10</td>
<td>13.1</td>
<td>1.69</td>
<td>1.16</td>
<td>30.44</td>
<td>0.84</td>
<td>1.69</td>
<td>3.35</td>
<td>3.35</td>
</tr>
<tr>
<td>Adult</td>
<td>17.5</td>
<td>1.17</td>
<td>1.01</td>
<td>36.08</td>
<td>0.99</td>
<td>1.17</td>
<td>0.72</td>
<td>0.72</td>
</tr>
<tr>
<td>Aged</td>
<td>21.8</td>
<td>1.60</td>
<td>0.91</td>
<td>34.75</td>
<td>1.15</td>
<td>1.60</td>
<td>1.21</td>
<td>1.21</td>
</tr>
</tbody>
</table>

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**TABLE 3**

<table>
<thead>
<tr>
<th>VOC</th>
<th>Km (mg/l)</th>
<th>Vmaxc (mg/h/kg)</th>
<th>Kfc (/h/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perchloroethylene(^a)</td>
<td>—</td>
<td>—</td>
<td>0.3</td>
</tr>
<tr>
<td>Trichloroethylene(^b)</td>
<td>0.25</td>
<td>11.0</td>
<td>—</td>
</tr>
<tr>
<td>Benzene(^c)</td>
<td>3.12</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Chloroform(^d)</td>
<td>0.25</td>
<td>6.8</td>
<td>—</td>
</tr>
<tr>
<td>Methylene chloride(^e)</td>
<td>0.40</td>
<td>4.0</td>
<td>2.0</td>
</tr>
<tr>
<td>MEK(^f)</td>
<td>0.63</td>
<td>5.4</td>
<td>4.1</td>
</tr>
</tbody>
</table>

\(^a\)Gargas et al. (1990).  
\(^b\)Medinsky et al. (1989).  
\(^c\)Thrall et al. (2002).

**Age-dependent scaling of metabolism.** The whole tissue Vmax used in the model can be estimated from *in vitro* data as follows:

\[ V_{max} = V_{max}(in vitro) \times Cmp \times VL, \]  
(1)

where Cmp and VL represent microsomal protein concentration and absolute liver volume, respectively (Krishnam and Andersen, 2001). Literature data reporting *in vitro* metabolic activity with standard substrates and microsomal protein measurements were used to adjust adult Vmax values to age-appropriate values. The following expression summarizes the adjustments for estimating age-appropriate Vmax values:

\[ V_{max,x} = Ra \times Rmp \times Rvl \times V_{max,\text{adult}} \]  
(2)

where \( x \) refers to PND10 or aged rat, and

\[ Ra = \frac{(CYP2E1 \text{ activity})_{x, \text{adult}}}{(CYP2E1 \text{ activity})_{x, \text{adult}}} \times \frac{Cmp}{Cmp_{\text{adult}}}, \quad \text{and} \quad Rvl = \frac{VL_{x}}{VL_{\text{adult}}} \]

The estimated age-appropriate values for \( Ra, Rmp, \) and \( Rvl \) are listed in Table 4. The products of these three scaling constants represent the total adjustment to adult Vmax values to account for age-dependent differences in CYP2E1 metabolism. In the case of perchloroethylene, the adjustment was applied to the first order metabolic rate constant (Kf) for CYP2E1 metabolism (Table 3).

Since the metabolism of methylene chloride also involves glutathione conjugation, a separate set of metabolic adjustment constants, designated as Rakf, Rlp, and Rvl, was applied to Kf values for the alternate metabolic pathway as follows:

\[ K_{f,x} = Rakf \times Rlp \times Rvl \times K_{f,\text{adult}} \]  
(3)

Rakf is similar to Ra, except that it applies to GST9 activity. As GST9 lacks activity toward the model GST substrate 1-chloro-2,4-dinitrobenzene (Meyer et al., 1991), studies reporting GST activity toward more specific GST substrates were used to adjust adult GST9 activity to age-appropriate values. For PND10, GST activity toward 1,2-epoxy-3-\( p \)-nitrophenoxypyropane, a relatively specific substrate for GST9, was used (Gregus et al., 1985). Rlp is similar to Rmp, except that it represents cytosolic protein per gram of liver. For the aged, Rakf was estimated using reported GST activity toward \( p \)-nitrobenzyl chloride (Chengelis, 1988a). In all cases, Rvl was the same as above. The estimated age-appropriate values for Rakf, Rlp, and Rvl are listed in Table 4. The products of Rakf, Rlp, and Rvl represent the total age-dependent adjustments for GST9-mediated metabolism of methylene chloride.

In this case, Rakf was assumed, due to lack of data, to be one for all of the ages and Rlp and Rvl were the same as those used for GST9 (Table 4).
Local sensitivity analysis was performed using the OptStat option of acslXtreme with normalization to parameter and response variable. In all cases, the perturbation of 1% was calculated and individually entered for each parameter to allow variation by the same proportion.

Steady-state analysis. Steady state was simulated by modeling constant exposure to 50 and 500 ppm VOC for 500 h. The time to reach 90% of steady-state levels was then estimated by examining the predicted venous concentration–time profile.

**RESULTS**

The VOCs examined in this study were first ranked according to lipophilicity and water solubility. Using fat:air partition coefficients (Mahle et al., 2005), perchloroethylene was ranked as the most lipophilic VOC, followed by trichloroethylene, benzene, chloroform, MEK, and methylene chloride. Although methylene chloride was ranked as the least lipophilic, it was MEK which exhibited the greatest water solubility (Poulin and Krishnan, 1996) (Fig. 2). Thus, perchloroethylene and MEK represent the two extremes among the selected VOCs for lipophilicity and water solubility, respectively, and their pharmacokinetic behaviors should differ accordingly.

In modeling the pharmacokinetics of the selected VOCs in the rat, it was assumed that this particular set of VOCs behaves as category 3 gases as described by US Environmental Protection Agency (EPA) inhalation reference concentration (RfC) guidelines (USEPA, 1994), causing effects due to systemic distribution and not undergoing deposition or metabolism in the alveolar region to any significant degree. Category 3 classification also entails rapid equilibration between inhaled air and the capillary blood leaving the lungs. Given this assumption, venous blood concentration was selected as one dose metric for initially examining age-dependent differences in internal dosimetry. Thus, 6 h inhalation exposures to 50 or 500 ppm VOC were simulated for a PND10, adult, or aged rat and the resulting venous concentration was predicted for 24 h. These simulated exposures span those reported in many chronic and subchronic animal toxicity studies for the selected VOCs (ATSDR, 2006).

### TABLE 4
Scaling Constants Used for the Metabolism of Different VOCs at Different Ages of the Rat

<table>
<thead>
<tr>
<th>Age</th>
<th>Enzyme activity (Ra or Rakf)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Liver fraction (Rmp&lt;sup&gt;b&lt;/sup&gt; or Rlp&lt;sup&gt;c&lt;/sup&gt;)</th>
<th>Liver volume (Rvl&lt;sup&gt;d&lt;/sup&gt;)</th>
<th>Total adjustment</th>
</tr>
</thead>
<tbody>
<tr>
<td>PND10</td>
<td>0.71&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.95&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.063&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.42</td>
</tr>
<tr>
<td>Aged</td>
<td>0.45&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.25&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.93&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.1</td>
</tr>
<tr>
<td>Glutathione transferase&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.89&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.063&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.013</td>
</tr>
<tr>
<td>PND10</td>
<td>1.66&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.84&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.93&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.7</td>
</tr>
<tr>
<td>Aged</td>
<td>1.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.84&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.93&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Note. The adult rat is the reference with values equal to 1.

<sup>a</sup>Ra or Rakf = Vmax(in vitro)<sub>PND10 or aged</sub>/ Vmax(in vitro)<sub>adult</sub>.

<sup>b</sup>Rmp = (microsomal protein content)<sub>PND10 or aged</sub>/(microsomal protein content)<sub>adult</sub>.

<sup>c</sup>Rlp = (cytosolic protein content)<sub>PND10 or aged</sub>/(cytosolic protein content)<sub>adult</sub>.

<sup>d</sup>Rvl = (Liver volume)<sub>PND10 or aged</sub>/(liver volume)<sub>adult</sub>.

<sup>e</sup>Applicable to all VOCs.

<sup>f</sup>Calculated from (Davies et al., 1976).

<sup>g</sup>Assumed to be the same as PND8 and calculated from (Yoon et al., 2006).

<sup>h</sup>Calculated from Table 1.

<sup>i</sup>Calculated from (Chengelis, 1988b).

<sup>j</sup>Applicable to methylene chloride only.

<sup>k</sup>Calculated from (Gregus et al., 1985).

<sup>l</sup>Calculated from (Chengelis, 1988a).

<sup>m</sup>Applicable to methyl ethyl ketone only.

<sup>n</sup>Due to lack of age-specific data, assume to be 1.

**Sensitivity analysis.** Local sensitivity analysis was performed using the OptStat option of acslXtreme with normalization to parameter and response variable. In all cases, the perturbation of 1% was calculated and individually entered for each parameter to allow variation by the same proportion.

**Steady-state analysis.** Steady state was simulated by modeling constant exposure to 50 and 500 ppm VOC for 500 h. The time to reach 90% of stead-state level was then estimated by examining the predicted venous concentration–time profile.

**FIG. 2.** Structures of VOCs examined in this study ranked by lipophilicity and water solubility.
The differences observed between the aged and adult rat at the end of a simulated 6 h exposure were not dramatic; generally venous concentrations varied slightly for all VOCs examined at 500 ppm. Figures 3A–F show the resulting plots for all of the VOCs analyzed and Tables 5 and 6 list the corresponding peak venous concentrations and area under the curve (AUC) values, respectively. The general trend exhibited by predicted peak venous levels was that the aged rat exhibited equal or slightly higher values in the case of benzene, chloroform, methylene chloride, and MEK, while slightly lower values were observed for the most lipophilic compounds analyzed, namely perchloroethylene and trichloroethylene. A similar trend was exhibited by AUC values. For comparison purposes, a lower inhalation exposure of 50 ppm was also simulated. As shown by Tables 5 and 6, the lower inhalation exposure did not affect peak venous blood levels or AUC values for the aged rat relative to the adult.

Sensitivity analysis identifies the parameters with the greatest impact on a model output. Local sensitivity analysis calculates sensitivity coefficients (SC) that reflect the change in model output relative to a given change in a particular parameter and the direction of the impact would be indicated.

**FIG. 3.** PBPK model simulations of venous concentration of (A) perchloroethylene, (B) trichloroethylene, (C) benzene, (D) chloroform, (E) methylene chloride, and (F) MEK. Simulations consisted of a typical inhalation exposure of 500 ppm VOC for 6 h for PND10, adult (2-month-old), and aged (2-year-old) rats using the PBPK model depicted in Figure 1. The adult and aged lines are superimposed in Figure 3F.
VOCs, especially at 500 ppm at which the effects of its water solubility become more apparent. For instance, the sensitivity of PB is negligible for loading of venous blood while QPI was observed at 50 ppm with respect to sensitivity to QGC, QCI, notably, the same general trend as with the other VOCs was predicted to be the parameter with the greatest impact at both modeled exposure levels. In addition, negative SCs were observed at 500 ppm for parameters associated with metabolism such as liver volume fraction (VLC), Vmax, and Kf (Fig. 5). Notably, the same general trend as with the other VOCs was observed at 50 ppm with respect to sensitivity to QGC, QCI, VSC, and PS.

**Aged versus Adult Rat Comparison: Exposure to Steady State**

Although the clearance of the simulated 6-h inhalation exposure is predicted to be essentially complete after 24 h (Figs. 3A–F), a pharmacokinetic difference noted between the aged and adult rat was the time to reach steady state. Steady state will be achieved when tissues become equilibrated with the inhaled VOC and the net pulmonary uptake is balanced by the inhaled VOC and the net pulmonary uptake is balanced by systemic clearance. In this study, steady state is being modeled as continuous inhalation, which is sometimes used experimentally (Kjellstrand et al., 1981), though less frequently than repeated daily dosing. Table 7 lists the approximate times to reach 90% steady-state venous levels of VOC following continuous inhalation exposure to 500 or 50 ppm. With the exception of MEK for which times are about equal, the results indicate that the adult rat reaches steady state faster at both simulated exposure levels when compared to the aged for all the VOCs examined. The greater percent of body fat (approximately 2-fold greater) in the aged rat appears to be responsible for the longer time to reach steady state, especially for the most lipophilic VOCs. Figures 6 and 6B show the simulations for...
perchloroethylene as the fat compartment and venous blood reach steady state, respectively. Thus, the 6-h time point represents a different phase in the venous concentration–time profile for the two ages. Indeed, even though higher peak venous concentrations of perchloroethylene and trichloroethylene are reached at 6 h in the adult at 500 ppm, it is the aged rat which achieves higher venous concentrations at steady state (Table 8). The underlying basis for the higher steady-state venous levels of the aged rat was attributed to PB which, with the exception of MEK, exhibited an age-dependent increase for all of the VOCs examined (Table 2). In the case of MEK, the comparable predicted results for the two ages are likely a reflection of the high water solubility of this compound (Figure 1) which overwhelms the impact of age-related differences in fat content on pharmacokinetics. Results of the sensitivity analyses for trichloroethylene and MEK at steady state are presented in Figures 4A and 5.

PND10 versus Adult Rat: 6 h Exposure

Previous studies on the effect(s) of age on human pharmacokinetics have concluded that the greater difference is between neonates/children (rather than aged) and adults (Clewell et al., 2004; Price et al., 2003). In an effort to examine if a similar trend would be exhibited by the rat, the pharmacokinetics of VOCs were simulated in PND10 rats and compared to results obtained with the adult. Simulation results of a 500 ppm VOC exposure for 6 h resulted in higher peak venous concentrations in PND10 animals for all the VOCs examined. Figures 3A–F show the predicted venous concentration–time profile for PND10 and the resulting peak venous concentrations and AUC values for all VOCs examined are listed in Tables 5 and 6, respectively.

In contrast to the aged rat, the higher venous concentrations predicted for PND10 cannot be attributed to PB since values for PND10 are lower than or about equal to adult values (Table 2). The combination of a higher QPI value (approximately 2.5-fold higher, Table 1) in PND10 animals and a much lower metabolic capacity overwhelm the opposing effect of a lower PB value,
resulting in higher venous levels at 6 h when compared to the adult rat. The reported specific activity of relevant metabolic enzymes and liver protein content (Table 4) do not account for the predicted much reduced metabolic capability of PND10 compared to the adult rat. It is primarily the PND10 liver size being approximately 16-fold smaller than that for the adult (Table 1, 0.62 g for PND10 vs. 9.8 g for adult) which results in a net metabolic capability for PND10 animals of less than 5% that of the adult. Thus, metabolism can be expected to play a much reduced role for PND10 in overall clearance at exposure levels where it represents a major clearance pathway for the adult rat. This is depicted in Figures 7A–D which show the predicted liver venous concentrations (CVL) and corresponding rates of metabolism for the two ages during the 6 h simulated exposure to 50 and 500 ppm trichloroethylene. In Figures 7A and 7B, predicted CVL for the adult rat far exceeds the $K_m$ of 0.25 mg/l for trichloroethylene at 500 ppm, while being significantly lower at 50 ppm. In the corresponding figure inserts, the adult rat metabolism in parallel exhibits first order metabolism at 50 ppm while being predominantly saturated at 500 ppm. On the other hand, the relatively low PND10 metabolism is predicted to be (or nearly be) saturated at both 50 and 500 ppm and mostly operating at predicted liver venous levels above the $K_m$ for trichloroethylene in both cases.

One of the most apparent consequences of the saturated metabolic capacity of PND10 at both exposure levels is that predicted venous concentrations of VOCs are likely to change directly in proportion with exposure level. Indeed, predicted venous levels and AUC values for PND10 (Tables 5, 6, and 8) exhibited nearly the same 10-fold change as exposure level, whereas a greater difference was observed for the adult rat due to the increased role of metabolism in systemic clearance at 50 ppm. This is clearly reflected in the PND10-to-adult ratios for venous levels and AUC values which exhibited an increase at 50 ppm as compared to 500 ppm, especially for highly metabolized VOCs such as trichloroethylene. The PND10-to-adult ratios did not show the same increase at 50 ppm for perchloroethylene, reflecting its slow metabolism (Tables 5, 6, and 8).

Overall, the PND10 liver is predicted to be less efficient per unit liver volume, in some cases by several folds, in metabolizing VOCs as compared to the adult. Table 9 lists the amount of VOC metabolized per unit liver volume by PND10 animals as compared to the other ages at 24 h following the simulated 6-h exposure to 500 or 50 ppm VOC. The slow metabolism of perchloroethylene is predicted to be even less efficient in PND10 animals as compared to the adult rat. Since some of the toxicity associated with VOCs is due to bioactivation to a toxic intermediate, it is not clear solely from this study if the lower metabolic capability of PND10 would result in less toxicity or a shift to a different type of toxicity such as CNS depression which is often associated with the parent compound. CNS depression may be particularly relevant since the PND10 brain not only represents a larger fractional volume in these animals, but also receives a more than 2-fold higher fractional blood flow when compared to the adult (Table 1). Thus, the
The concentration of VOC achieved in the brain is another dose metric of toxicological relevance. As indicated in Table 10, the predicted brain levels reached in PND10 rats at 6 h were predicted to parallel venous levels. In particular, MEK is predicted to reach brain levels more than 25-fold higher than those of the adult as similarly observed for venous levels.

Sensitivity analysis of peak venous levels achieved in PND10 identified PB and QPI as being the most important

### TABLE 9
Predicted Amount of VOC Metabolized per Unit Liver Volume (mg/l) for Different Ages of the Rat at 24 h following a 50 or 500 ppm Inhalation Exposure for 6 h

<table>
<thead>
<tr>
<th>VOC</th>
<th>PND10</th>
<th>Adult</th>
<th>Aged</th>
<th>Aged/adult</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perchloroethylene</td>
<td>500 ppm</td>
<td>12</td>
<td>104</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>50 ppm</td>
<td>1.2</td>
<td>10.4</td>
<td>9.7</td>
</tr>
<tr>
<td>Trichloroethylene</td>
<td>500 ppm</td>
<td>635</td>
<td>2900</td>
<td>3785</td>
</tr>
<tr>
<td></td>
<td>50 ppm</td>
<td>385</td>
<td>420</td>
<td>506</td>
</tr>
<tr>
<td>Benzene</td>
<td>500 ppm</td>
<td>273</td>
<td>1530</td>
<td>1889</td>
</tr>
<tr>
<td></td>
<td>50 ppm</td>
<td>81</td>
<td>195</td>
<td>236</td>
</tr>
<tr>
<td>Chloroform</td>
<td>500 ppm</td>
<td>317</td>
<td>2004</td>
<td>2536</td>
</tr>
<tr>
<td></td>
<td>50 ppm</td>
<td>215</td>
<td>386</td>
<td>458</td>
</tr>
<tr>
<td>Methylene chloride</td>
<td>500 ppm</td>
<td>163</td>
<td>1257</td>
<td>1745</td>
</tr>
<tr>
<td></td>
<td>50 ppm</td>
<td>101</td>
<td>259</td>
<td>305</td>
</tr>
<tr>
<td>MEK</td>
<td>500 ppm</td>
<td>1461</td>
<td>2557</td>
<td>2982</td>
</tr>
<tr>
<td></td>
<td>50 ppm</td>
<td>376</td>
<td>286</td>
<td>333</td>
</tr>
</tbody>
</table>

### TABLE 10
Predicted Peak Brain Concentrations (mg/l) of VOCs for Different Ages of the Rat following a 50 or 500 ppm Inhalation Exposure for 6 h

<table>
<thead>
<tr>
<th>VOC</th>
<th>PND10</th>
<th>Adult</th>
<th>Aged</th>
<th>Aged/adult</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perchloroethylene</td>
<td>500 ppm</td>
<td>70</td>
<td>91</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td>50 ppm</td>
<td>7.0</td>
<td>9.1</td>
<td>7.8</td>
</tr>
<tr>
<td>Trichloroethylene</td>
<td>500 ppm</td>
<td>28</td>
<td>23</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>50 ppm</td>
<td>2.6</td>
<td>1.0</td>
<td>1.2</td>
</tr>
<tr>
<td>Benzene</td>
<td>500 ppm</td>
<td>15</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>50 ppm</td>
<td>1.5</td>
<td>0.9</td>
<td>1.0</td>
</tr>
<tr>
<td>Chloroform</td>
<td>500 ppm</td>
<td>23</td>
<td>22</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>50 ppm</td>
<td>2.2</td>
<td>0.7</td>
<td>0.8</td>
</tr>
<tr>
<td>Methylene chloride</td>
<td>500 ppm</td>
<td>16</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>50 ppm</td>
<td>1.5</td>
<td>0.4</td>
<td>0.5</td>
</tr>
<tr>
<td>MEK</td>
<td>500 ppm</td>
<td>201</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>50 ppm</td>
<td>18.5</td>
<td>0.7</td>
<td>0.83</td>
</tr>
</tbody>
</table>
parameters during the loading phase, as similarly observed for the adult. This analysis also identified negative sensitivity with respect to the volume and partition coefficient of slowly perfused compartment during the loading phase (Fig. 8). This particular compartment exhibited an age-dependent decrease in volume and blood flow (Table 1) and is a difference in physiology/anatomy across life stages, potentially leading to pharmacokinetic differences. Furthermore, the partition coefficient for muscle, used for the slowly perfused compartment, was significantly higher in PND10 animals compared to the other ages (Table 2), inversely impacting the venous levels achieved in these animals.

**PND10 versus Adult: Exposure to Steady State**

In general, the PND10 rat was predicted to reach equal or higher steady-state venous levels as the adult at both simulated exposure levels (Table 8), reflecting the combination of a higher QPI, relatively low metabolic capacity, and smaller fat volume of this age. With the exception of MEK, the PND10 rat was also predicted to achieve steady-state venous levels faster than the adult rat at 500 ppm, and, with the exception of perchloroethylene and MEK, within the simulated 6-h exposure (Table 7). Figures 6A and 6B show the fat and venous concentration–time profile of perchloroethylene, respectively, to steady state for PND10 as compared to the adult. Similarly to the 6-h exposure results, the PND10-to-adult ratios were predicted to increase at the lower inhalation exposure of 50 ppm, reflecting the dose-dependent differences in metabolism for the adult rat. Furthermore, in contrast to PND10 for which times to reach 90% steady-state venous levels were predicted to be the same for both exposure levels, the adult rat was predicted to reach such levels faster at 50 ppm due to the major role of metabolic clearance at these low levels of exposure. This dose-dependent effect on metabolism is absent for perchloroethylene whose slow metabolism is unaffected by the lower exposure level.

Sensitivity analysis at steady state yielded results consistent with venous concentrations being sensitive to only PB at both exposure levels. Figure 8 depicts the sensitivity analysis results for the highly metabolized VOC, trichloroethylene at steady state. Similar results were obtained for the other VOCs. Thus, in contrast to the adult rat, steady-state venous levels of VOCs predicted for PND10 exhibited sensitivity exclusively to PB.

**DISCUSSION**

The analyses of this inhalation-dosimetry modeling study found relatively small differences in predicted systemic venous concentrations of VOCs across three ages of the rat. In general, the magnitude of the differences observed (generally less than 2-fold) is consistent with previous reports involving humans. For instance, Clewell et al. (2004), reported that predictions of pharmacokinetic dose metrics across human life stages were within a factor of 2 for a variety of environmental chemicals including VOCs. Similarly, Price et al. (2003) reported predicted blood concentrations of VOCs in children to be about 1.5 greater than in adults. Our simulation results with the rat are also consistent with previous reports indicating the greater pharmacokinetic difference being between neonate and adult rather than between the aged and adult (Clewell et al., 2004).

Among the various pharmacokinetic parameters analyzed for age-dependent effects on venous concentration, PB was identified as a critical parameter. PB exhibited an age-dependent increase (Table 2), and was identified as a determinant factor in the higher venous concentrations achieved in the aged rat as compared to the adult, especially at steady state. It should be emphasized that the age-dependent increase in PB observed in the rat may not be pertinent to other species. For instance, humans and mice reportedly do not exhibit the age-dependent increase in PB seen in the rat, at least not for some of the VOCs examined in this study (Mahle et al., 2005; Thomas et al., 1996). Studies of several volatile anesthetics in human blood found less than 20% differences in PB across ages (Lerman et al., 1984; Malviya and Lerman, 1990). The increase in rat PB may be the result of reported age-related increases in total plasma cholesterol and different lipid composition of lipoproteins, resulting in higher solubility of lipophilic compounds such as VOCs in these species (Bravo et al., 1996; Masella et al., 1995). Other parameters that likely affect the predicted differences between the aged and adult rat, particularly for the most lipophilic VOCs, include the volume and blood flow of the fat compartment, which are predicted to be of more impact for the most lipophilic VOCs. The impact of PB and fat-associated parameters on venous concentration is depicted in Figure 9.

In the case of PND10, the higher venous concentrations of VOC were mainly attributed to a higher QPI and a lower...
metabolic capacity of these animals. The impact of each of these parameters on venous concentration is also depicted in Figure 9. The higher QPI has been emphasized in previous studies involving early life stages. Clewell et al. (2004) reported minute ventilation rates normalized to BW of children being higher than adults by 40–50%. Similarly, Sarangapani et al. (2003) attributed a likely increased risk of chemical exposure to a higher ventilation rate per unit BW of children. The lower metabolic capacity of PND10 rats is also consistent with previous studies involving early life stages. In contrast to previous studies for humans, however, which have attributed the lower metabolic capacity to immature xenobiotic metabolizing enzyme systems (Clewell et al., 2004), our analysis indicates that the lower metabolic capacity of PND10 rats is mainly due to a much smaller liver size compared to the adult. Since substantial liver growth and development of enzyme systems (e.g., CYP2E1) occur by PND10, much reduced metabolic activity can be expected for even younger ages.

Among the VOCs examined in this study, MEK represents a distinct case due to its relatively high water solubility which predominantly dictates its pharmacokinetic behavior. Predicted venous levels for this particular VOC were more than 25-fold greater for PND10 compared to the other ages. The high water solubility along with the combination of the low metabolic capacity and higher QPI of PND10 were identified as the critical determinants for the much higher venous levels.

The results of this modeling effort are based, due to lack of age-appropriate data, on several assumptions which need to be emphasized. First, the estimation of QC for PND10 was based on extrapolation to younger ages using nonlinear regression analysis of QC versus BW with the earliest data collected at PND21. Although a reasonably good fit ($r^2 = 0.82$) was obtained, it is unclear how QC actually changes in the early ages. In addition, the QP for the adult and aged rat is being assumed to be equal to QC. Such a relationship has been observed for humans at rest, but it is unclear if the same holds for other species. In the case of PND10, QP was independently measured (Bandla et al., 1999) and the results indicate that QP is nearly equal to our predicted QC (Table 1). QC and QP are critical physiological parameters for PBPK modeling and more research is needed on how they change as a function of age; no data on QC exist prior to weaning.

There is also the assumption that the CYP-mediated metabolism of these VOCs is exclusively catalyzed by CYP2E1. Depending on concentration, other CYP isoforms may be involved. For instance, Nakajima et al. (1992) reported several low affinity CYP isoforms in the metabolism of trichloroethylene, which included CYP2C11 and CYP2B1. There is also the potential for induction of CYP enzymes by VOCs, which would be especially relevant in the case of repeated daily exposures. Induction of CYP enzymes in neonatal rats has been reported in the case of musk xylene and toluene (Hansson et al., 1985; Suter-Eichenberger et al., 2000). In such studies, small but significant increases in liver size were also reported, which are likely to affect the metabolic capability of neonatal rats, as demonstrated in this particular study for PND10.

In the case of MEK with a secondary reductive metabolic pathway, the specific activity for its reductive metabolism was assumed to be the same for all of the ages modeled. This assumption does not affect PND10 whose metabolic capability is already significantly diminished due to a relatively smaller VLC, but it does affect significantly the predicted results for the aged. If the aged specific activity were 50% that of the adult, peak venous levels are predicted to increase by about 40%. Similarly, peak venous levels are predicted to be 40% lower if the specific activity for the aged was 2-fold greater than that of the adult.

Our parameter estimation did not adjust the slowly perfused compartment to maintain a constant sum of tissue masses across ages. Instead, the actual tissue masses reported were used to account for this large compartment. Although this approach does not significantly affect model predictions, it is a feature of the model that should be noted.

The difference in dose metric observed at 6 h versus steady state may have significant implications on the interpretation of dosimetry in toxicity studies. In particular, on a single day, PND10 rats are predicted to achieve steady-state levels faster as compared to the adult and aged rats. However, preliminary simulation studies indicate that it is unlikely that the young animal will approximate steady state with repeated daily 6-h exposures with any of the VOCs examined, due to more rapid clearance when compared to the other ages.

To date, tissue dosimetry–based risk assessments for VOCs using PBPK models have assumed that the dose metrics predicted for young adult animals were also appropriate for other ages (e.g., Integrated Risk Information System assessments for methylene chloride and vinyl chloride; Andersen et al., 1987; USEPA, 2006). The results presented here for venous and brain levels as well as total amount of VOC metabolized per unit liver volume indicate that this is a reasonable approach for these particular dose metrics. However, dose metrics for specific metabolites that are more water soluble, less volatile, and cleared by other pathways such as urinary or biliary elimination may show different age-related changes. It should be noted that this study models only inhalation

![Diagram](https://academic.oup.com/toxsci/article-abstract/98/1/43/1662409/1862408)
exposure, although studies with PND10 rats would likely also involve lactational exposure. Pharmacokinetic modeling studies of exposure by gavage, feeding, drinking, and lactational transfer are needed to explore age-dependent dosimetry changes that might arise in one- and two-generation toxicity studies, developmental, and neurotoxicity studies, all of which involve multiple life stages.

REFERENCES


