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COMPARING RESPIRATORY-TRACT AND HEPATIC EXPOSURE–DOSE RELATIONSHIPS FOR METABOLIZED INHALED VAPORS: A PHARMACOKINETIC ANALYSIS

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Inhaled vapors that are metabolized locally in the respiratory-tract tissues and systemically in the liver and other organs have different dose-response relationships at the portal of entry compared to systemic target organs. For instance, inhaled chloroform and styrene cause cytotoxicity in the nasal cavity at concentrations much lower that those causing hepatic or renal toxicity. Here, we develop a physiologically based pharmacokinetic (PBPK) model that incorporates a multicompartment, unidirectional flow description of the respiratory tract within a whole-body model in order to estimate both respiratory tract and hepatic metabolism. We then use this model to study the difference in exposure–dose relationship between the respiratory-tract tissues and the liver. The integrated PBPK model confirms that for soluble vapors the exposure–dose curve for metabolism in respiratory-tract tissue will be shifted dramatically to lower concentrations compared to the exposure–dose relationship in systemic organs. This behavior is the result of direct air to tissue equilibration at the portal of entry while other systemic tissues only respond to concentrations in the blood. For cases where metabolism/metabolites of inhaled vapors produce local toxicity, portal of entry effects are expected at lower concentrations and, in general, will be the limiting response for setting reference concentrations (RfCs) for many compounds. The difference in dose-response relationships for metabolism in the respiratory tract versus systemic organs depends on blood/air and blood/tissue partition coefficients and on the degree of systemic extraction of the metabolized vapors.

Improved histopathological evaluation techniques in the upper and lower respiratory-tract tissues following inhalation toxicity studies have shown that a wide variety of compounds cause selective damage to epithelial tissues in these airways. Among the large set of compounds causing these responses are organic acids, organic esters, and several hydrocarbons

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and chlorocarbons that are metabolized to reactive intermediates via the mixed-function monooxygenase system (e.g., styrene, chloroform, and ethyl acrylate) (Christoph, 1998; Cruzan et al., 1997; Templin et al., 1996). Inhaled vapors that are highly water soluble (e.g., some organic esters and especially the organic acids) are characterized by high degree of extraction in the nasal cavity and show little systemic accumulation. These compounds predominantly cause contact-site toxicity and show an anterior-to-posterior gradation in lesion intensity and severity along the respiratory tract (Bogdanffy et al., 1994; Miller et al., 1981). On the other hand, moderately to poorly water soluble vapors (e.g., chloroform, methylene chloride, trichloroethylene, styrene, naphthalene, and butadiene) are not efficiently extracted in the extrathoracic region and penetrate the nasal cavity and conducting airways to reach the gas-exchange region. Such compounds have a potential to accumulate in the blood and can cause toxicity both at the portal of entry and in remote sites such as the liver and kidney (Cruzan et al., 1997; Larson et al., 1996; Templin et al., 1996).

Subchronic and chronic inhalation studies have shown that these inhaled compounds elicit similar dose-response for toxicity in systemic organs such as the liver and kidney. However, the dose-response for toxicity in portal of entry tissues and systemic organs differ (Cruzan et al., 1997; Templin et al., 1996). Toxicity in cells in the respiratory tract usually occurs at lower inhaled concentrations than do the effects in the systemic tissues (Cruzan et al., 1997; Templin et al., 1996). The differences in dose-response between systemic organs and respiratory tract tissues could be due to differences in tissue sensitivity or due to pharmacokinetic differences in delivered dose. It is instructive to evaluate the exposure–dose relationship for inhaled volatiles and their metabolic products at the portal of entry and in systemic organs to evaluate the reasons for the differences in dose-response behavior for these tissues. In this article we assess differences in expected exposure–dose curves for metabolism in the portal of entry versus remote site (i.e., respiratory tract tissue versus the liver) for inhaled vapors in rodents using a physiologically-based pharmacokinetic (PBPK) model. The PBPK model used in this study is based on previously published and validated models (Corley et al., 1990; Ramsey & Andersen, 1984). These PBPK models have been extended to include nasal cavity and conducting airways in this article. This revised PBPK model provides for unidirectional flow pathways through the several regions of the respiratory tract, with delivery of xenobiotics to epithelial tissues from both ventilation and blood perfusion. In addition to the model-based analysis, we have also provide a theoretical formulation to estimate the differential exposure–dose relationships in the portal of entry tissues and systemic organs using simple steady-state assumptions. These theoretical formulations result in easy-to-use closed form expressions that can provide results akin to PBPK model-based simulations and are helpful in understanding the dominant parameters affecting tissue dose metrics.

METHODS

Analysis of Animal Bioassay Data

Templin et al. (1996) exposed F344 rats to airborne concentrations of 0, 2, 10, 30, 90, or 300 ppm chloroform 6 h/day, 7 days/wk for 13 wk to study the exposure-response relationship for chloroform-induced lesions and regenerative cell proliferation in rats. They identified the kidney, liver, and nasal cavity as the primary target organs. Chloroform-induced cytolethality and regenerative cell proliferation in the liver was seen only at the highest exposure concentration of 300 ppm in male rats. This dose response was in contrast to the nasal tissue, where histological alterations and an increased unit length labeling index were found at concentrations as low as 10 ppm (Table 1). Similarly, in a subchronic inhalation study exposing CD-1 mice to styrene vapors at 0, 50, 100, 150, and 200 ppm for 6 h/day, 5 days/wk for 13 wk Cruzan et al. (1997) observed significant liver toxicity only at 150 ppm, whereas treatment related, statistically significant histopathological changes in the nasal cavity were observed at the lowest concentration of 50 ppm in female mice (Table 1). A recent study has shown that chronic 2-yr inhalation exposure to styrene at 0, 20, 40, 80, and 160 ppm for 6 h/day, 5 day/wk causes a 100% nonneoplastic response in the conducting airways (Clara-cell-rich bronchiolar region) in CD-1 mice starting from the lower exposure concentration of 20 ppm (Cruzan et al., 2001). No statistically significant response in the liver were observed for the whole range of exposure concentrations in this study (Cruzan et al., 2001).

PBPK Model Structure

A number of PK models have been developed to describe the systemic disposition and metabolism of inhaled vapors in tissues throughout the body.

TABLE 1. Summary of Nasal and Hepatic Lesions in Male F-344 Rats Exposed to Chloroform Vapors for 6 h/day, 7 days/wk for 13 wk (as Reported in Templin et al., 1996) and in Female CD-1 Mice Exposed to Styrene Vapors for 6 h/day, 5 days/wk for 13 wk (as Reported in Templin et al., 1996)

Male F-344 rats exposed to chloroform			Female CD-1 mice exposed to styrene		
Exposure (ppm)	Nasal lesions	Liver lesions	Exposure (ppm)	Nasal lesions	Liver lesions
0	0/10	1/15	0	0/10	0/10
2	10/10	3/15	50	4/10	0/10
10	10/10	0/15	100	10/10	0/10
30	10/10	2/15	150	10/10	3/10
90	10/10	14/15	200	8/8	8/8
300	10/10	15/15	—	—	—

Note. Nasal lesions include general atrophy of the ethmoid turbinates, focal hyperplasia, and respiratory metaplasia. Hepatic lesions include focal loss of hepatocytes, hepatocyte necrosis, and centrilobular aggregates of siderophages.

However, most of these models have described the respiratory tract as a single uniform tissue compartment that passively aids in gas exchange (Corley et al., 1990; Ramsey & Andersen, 1984). Here, we have constructed a multicompartment respiratory tract model that also provides metabolism in these tissues and assesses target organ toxicity through regional metabolism within the respiratory tract (Figure 1). Apart from the respiratory tract, the PBPK model also has compartments for the liver, fat, and richly and poorly perfused tissues. Based on anatomical location and function, the respiratory tract is divided into three regions: nasal cavity, conducting airways (i.e., non-

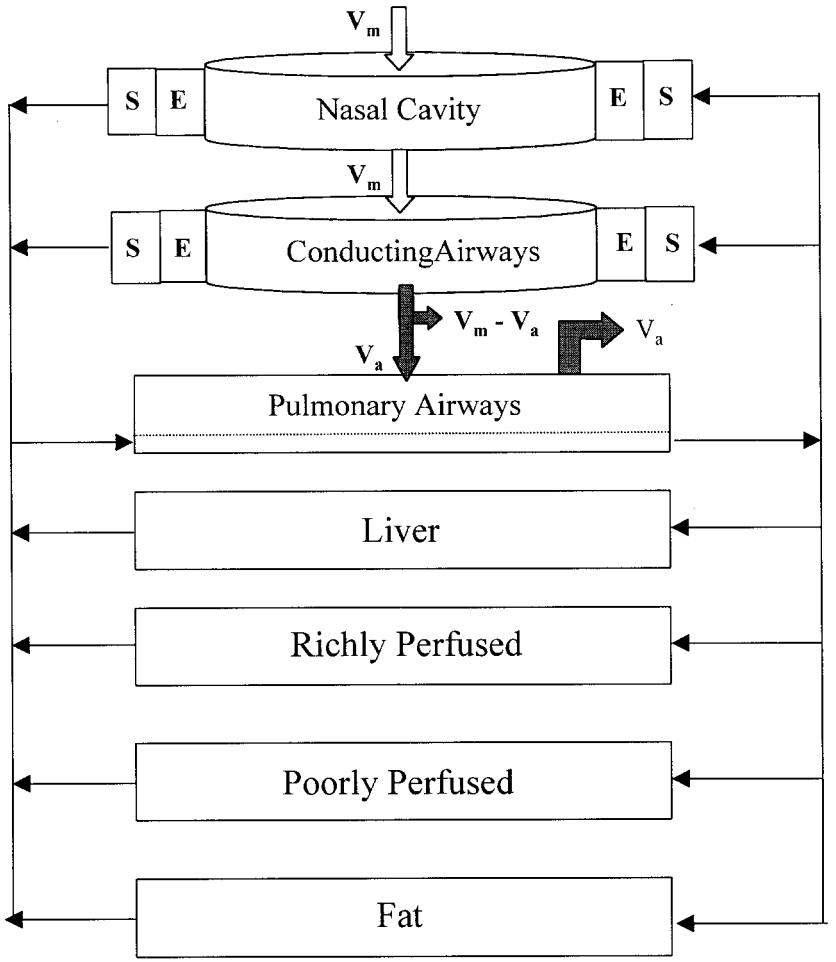

FIGURE 1. Schematic of the PBPK model showing the three-compartment description of the respiratory tract (portal of entry) and the other systemic tissue compartments. The respiratory tract is divided into nasal cavity, conducting airways, and pulmonary airways (i.e., gas-exchange region). The nasal cavity and conducting airways have a three-layered structure comprising the lumen, epithelial cell layer (E), and submucosal layer (S) perfused by arterial blood. Airflow is unidirectional. Exhaled air is a mixture of alveolar air and air leaving the conducting airways.

TABLE 2. Mass Balance Equations to Model Uptake of Inhaled Volatiles in the Nasal Cavity, Conducting Airway, Pulmonary Airway and Hepatic Tissue

Mass balance in the anterior respiratory-tract compartments:

Airway lumen: $$V_l \frac{dC_l}{dt} = \dot{V}_m(C_{in} - C_l) - K_{at}\left(C_l - \frac{C_t}{H_{t:a}}\right)$$

Epithelial layer: $$V_t \frac{dC_t}{dt} = K_{at}\left(C_l - \frac{C_t}{H_{t:a}}\right) - K_{tb}\left(\frac{C_t}{H_{t:b}} - \frac{C_x}{H_{t:b}}\right) - \frac{V_{max}C_t / H_{t:b}}{K_m + C_t / H_{t:b}}$$

Submucosal layer: $$V_x \frac{dC_x}{dt} = K_{tb}\left(\frac{C_t}{H_{t:b}} - \frac{C_x}{H_{t:a}}\right) - \dot{Q}_x(C_{art} - C_x / H_{t:b})$$

Mass balance in the gas-exchange region of the lung:

$$\dot{V}_a(C_l - C_{art} / H_{b:a}) = \dot{Q}_c(C_{art} - C_{ven})$$

Mass balance in the liver:

$$V_{li} \frac{dC_{li}}{dt} = Q_{li}(C_{art} - C_{li} / H_{t:b}) - \frac{V_m C_{li} / H_{t:b}}{K_m + C_{li} / H_{t:b}}$$

Overall mass balance in the system:

$$\dot{Q}_c C_{ven} = \dot{Q}_{li} C_{li} + \dot{Q}_f C_f + \dot{Q}_{rt} C_{rt} + \dot{Q}_{pt} C_{pt} + \dot{Q}_x C_x$$

Note. C_l, C_t, C_x—species concentration in lumen, tissue, and submucosa, respectively. V_l, V_t, V_x—lumen, tissue, and submucosa compartment volumes, respectively. $\dot{Q}_c$, $\dot{Q}_x$, $\dot{Q}_{li}$—cardiac output, respiratory-tract, and hepatic blood flow rates, respectively. $\dot{Q}_f$, $\dot{Q}_{rt}$, $\dot{Q}_{pt}$—blood flow rate to fat, richly, and poorly perfused tissues, respectively. $\dot{V}_m$, $\dot{V}_a$—minute ventilation and alveolar ventilation rates, respectively. K_{lt}, K_{lx}—lumen:tissue and tissue:blood mass transfer coefficient, respectively. H_{ta}, H_{bt}—tissue:air and blood:tissue partition coefficient, respectively. C_{art}, C_{ven}, C_{li}—arterial blood, venous blood, and liver concentration, respectively. C_f, C_{rt}, C_{pt}—venous blood concentration exiting fat, richly, and poorly perfused tissues. V_{max}, K_m—capacity and affinity for metabolism in liver or respiratory-tract tissue.

gas-exchange region of the lung), and pulmonary airways (i.e., gas-exchange region of the lung). The pulmonary airway compartment achieves gas exchange between arterial blood and inhaled air. Arterial blood flowing out of the pulmonary airways transports the absorbed volatile to the anterior portions of the respiratory tract (i.e., nasal cavity and conducting airways), fat, liver, richly perfused, and poorly perfused tissue compartments. The venous blood exiting each compartment is equilibrated with the venous flow exiting the remaining tissue compartments. The mixed venous blood returns to the pulmonary airways at a flow rate equal to the cardiac output. The governing equations for the systemic compartments have been derived elsewhere (Corley et al., 1990; Ramsey & Andersen, 1984) and are only summarized here in Table 2.

In the PBPK model, all the compartments are represented by a single homogeneous tissue structure except for the anterior respiratory-tract tissues. The nasal cavity and the conducting airways form the portal of entry

for inhaled compounds and have a complex anatomy. Furthermore, unlike systemic organs, the respiratory-tract tissues are exposed to the inhaled chemical from both the air phase and blood. To include the various processes that transport the volatile chemicals across the anterior respiratory tract compartments these regions are further divided into a three-layered substructure as originally proposed by Morris and coworkers (Morris et al., 1993) (Figure 1). The three layers consist of a lumen, epithelial cell layer, and a submucosal tissue layer. Inhaled air ventilates the lumen of these two airway compartments. Chemical in the lumen is available for uptake into the epithelial lining tissues or passage to the subsequent airway compartment. The submucosal tissue layer has blood perfusion and clears the absorbed volatiles from the airway compartments. The epithelial cell layer, which usually is the target site for toxicity, contains enzymes for activation and detoxication of the inhaled compound. The model assumes unidirectional ventilation in the respiratory tract. Airflow that exits the nasal cavity enters into the conducting airways. Only a portion of the volumetric flow (V_m) in the nasal cavity and conducting airways ventilates the alveolar region (V_a). The remainder of the flow stream ($V_m - V_a$), equivalent to dead space ventilation, exits the respiratory tract at the lumen concentration from the conducting airways (Figure 1). Analytical expressions that describe the mass transport of the inhaled chemical in the respiratory-tract tissue compartments have been derived elsewhere (Bogdanffy et al., 1999b) and are summarized in Table 2. The model was coded using ACSL (Advanced Continuous Simulation Language, Aegis, Inc., Huntsville, AL).

The PBPK model was parameterized for the mouse and the rat (Table 3). The various physiological parameters in this model, such as tissue volumes, ventilation rate, cardiac output, and the blood perfusion rates to the various tissue compartments (i.e., liver, fat, richly perfused, poorly perfused tissue) were obtained from Brown et al. (1997). Physiological parameters specific to the respiratory tract compartments such as tissue surface area and blood perfusion rate were obtained from the literature for both mouse and rat (Bogdanffy et al., 1998; Menache et al., 1997; Stott et al., 1983, 1986). The epithelial tissue thickness and air-phase mass transfer coefficients (MTC) were obtained from literature for the rat (Bogdanffy et al., 1998; Frederick et al., 1998) and the corresponding values in the mouse were assumed to be same as in the rat. The liquid-phase MTC, which determines the chemical flux from the interface into the tissue, was estimated as a ratio of liquid-phase chemical diffusivity by half the tissue thickness (i.e., diffusion length). The composite air-to-tissue MTC, which determines the net chemical flux from the lumen into the tissue, was calculated as a function of the air-phase MTC, liquid-phase MTC, and the corresponding tissue:air partition coefficient (U.S. EPA, 1994). Chemical-specific parameters such as blood:air partition coefficients, tissue:blood partition coefficients, and kinetic constants (V_{max}, K_m) for hepatic P-450 activity reported in Table 4 were obtained from

TABLE 3. List of Physiological Model Parameters in Mouse and Rat (Brown et al., 1997)

Parameter	Units	Mouse	Rat	Reference
Body weight	g	25	250	(Brown et al., 1997)
Fractional weight of liver	% BW	5.5	4	Same
Fractional weight of richly perfused organ	% BW	6	6	Same
Fractional weight of poorly perfused organ	% BW	63	65	Same
Fractional weight of fat	% BW	7	6.5	Same
Minute ventilation[a]	ml/min	43.5	198	Same
Cardiac output	ml/min	14	110	Same
Blood flow to liver	% CO	25	25	Same
Blood flow to richly perfused organ	% CO	50	50	Same
Blood flow to poorly perfused organ	% CO	17.5	15	Same
Blood flow to fat	% CO	6	7	Same
Blood flow to nasal cavity	% CO	1	1	(Stott et al., 1983, 1986)
Blood flow to conducting airways	% CO	0.5	2	(Brown et al., 1997)
Nasal cavity surface area	cm^2	2.7	13.2	(Menache et al., 1997)
Conducting airway surface area	cm^2	8.9	48.3	(EPA, 1994)
Pulmonary airway surface area	cm^2	500	3400	Same
Nasal cavity tissue thickness	cm	0.005	0.005	(Bogdanffy et al., 1998)
Conducting airway tissue thickness	cm	0.0025	0.0025	Same
Nasal cavity mass transfer coefficient	cm/min	0.2	0.2	Computed[b]
Conducting airway mass transfer coefficient	cm/min	0.4	0.4	Same

[a]Alveolar ventilation is set at 67% of minute ventilation.

[b]Composite mass transfer coefficient (MTC) is computed based on gas-phase and liquid-phase MTC values. Liquid-phase MTC is given as a ratio of liquid diffusivity to mucus thickness.

TABLE 4. List of Chemical Specific Parameters for Mouse and Rat Based on Published Results from Corley et al. (1990) for Chloroform and Ramsey and Andersen (1984) for Styrene (ST)

Chemical	Parameter	Unit	Mouse	Rat	Reference
ST	Blood:air PC	—	40	40	(Ramsey & Andersen, 1984)
	Rapid perfused:blood PC	—	1.3	1.3	Same
	Poorly perfused:blood PC		1.3	1.3	Same
	Liver:blood PC	—	2	2	Same
	Fat:blood PC	—	87	87	Same
	Hepatic P-450 activity (V_{max})	nmol/min/ml	200	100	(Mendrala et al., 1993)
	Nasal P-450 activity (V_{max})	nmol/min/ml	200	100	Estimate
	P-450 affinity (K_m)	nmol/ml	10	10	(Mendrala et al., 1993)
CL	Blood:air PC	—	21	21	(Corley et al., 1990)
	Rapid perfused:blood PC	—	0.9	1	Same
	Poorly perfused:blood PC	—	0.62	0.67	
	Liver:blood PC	—	0.9	1	Same
	Fat:blood PC	—	11.5	9.7	Same
	Hepatic P-450 activity (V_{max})	nmol/min/ml	227.6	35	Same
	Nasal P-450 activity (V_{max})	nmol/min/ml	227.6	35	Estimate
	P-450 affinity (K_m)	nmol/ml	2.95	4.5	(Corley et al., 1990)

Corley et al. (1990) for chloroform and Ramsey and Andersen (1984) for styrene. Tissue:blood partition coefficient in the respiratory tract tissues was assumed to be same as those reported for the rapidly perfused tissues. P-450 activity in the nasal tissues have been shown to be high and comparable to hepatic activity on a unit tissue volume basis (Marini et al., 1998; Sarkar 1992; Thornton-Manning & Dahl, 1997). Hence the P-450 activity and enzyme affinity toward chloroform and styrene metabolism in the nasal cavity were assumed to be equal to the corresponding hepatic values on a per cell basis in those cells that express the activating enzymes.

Theoretical Formulation

The relative differences in dose-response curves for respiratory-tract and liver responses to metabolized volatiles are governed by the ratio of the achieved free tissue concentrations in nasal and liver tissues. Using a steady-state analysis, theoretical formulations were derived to compute free tissue concentrations in the respiratory-tract compartments and liver for a given exposure concentration of the inhaled volatile. These analytical expressions for estimating the free concentration of inhaled volatiles in the respiratory tract and liver tissues are valid at low exposure concentrations when hepatic metabolism is not saturated. The same analytical expressions can also be used to estimate exposure concentrations that would result in a given free concentration of the chemical in nasal and liver tissues. In contrast to complex PBPK models, theoretical formulations result in easy-to-use closed form expressions that can help understand the dominant parameters affecting tissue dose metrics and may serve as useful indicators of trends in organ-specific toxicity based on pharmacokinetic differences.

For inhaled compounds that are metabolized at the portal of entry as well as the liver, the metabolic capacity of the respiratory-tract tissues can be ignored when computing the arterial blood concentration because the tissue volume of the nasal cavity is small compared to liver tissue. Furthermore, for inhaled volatiles with relatively low blood:air partition coefficient and consequently low extraction in the anterior regions of the respiratory tract, such as chloroform and styrene (Morris, 2000), the concentration reaching the alveolar region can be approximated by the inhaled concentration. Hence, mass balance in the whole animal stipulates that the net uptake of the chemical from the alveolar region is equal to the hepatic clearance (Eq. 1). Furthermore, the rate of loss of the compound from the blood perfusing the liver is equal to its metabolic clearance (Eq. 2).

Overall mass balance:

$$\dot{V}_a\,(C_{in} - C_{art}/H_{ba}) = Cl_{li}C_{vl} \tag{1}$$

Liver compartment:

$$Q_{li}\,(C_{art} - C_{vl}) = Cl_{li}C_{vl} \tag{2}$$

Here Q_{li} is the hepatic blood flow and Cl_{li} is intrinsic hepatic metabolic clearance at low dose given as a ratio of metabolic capacity to metabolic affinity ($V_{li}V_{max}/K_m$) in the liver. The rest of the symbols are as defined in Table 1. Rearranging these two equations, arterial blood concentration (C_{art}) and the hepatic venous concentration or the free concentration in the liver (C_{vl}) can be represented in terms of system-independent parameters and the inhaled exposure concentration:

$$C_{art} = \dot{V}_a C_{in} \Big/ \left[\frac{V_{max}/K_m Cl_{li}}{\dot{Q}_{li} + C_{LI}} + \frac{\dot{V}_a}{H_{b:a}} \right] \text{ and } C_{vl} = C_{LI} = \left(\frac{\dot{Q}_{li}}{\dot{Q}_{li} + Cl_{li}} \right) C_{art} \qquad (3)$$

Assuming that the inhaled volatiles are in equilibrium with the respiratory tract tissues, the free concentration in these tissues can be approximated to $C_{RT} = H_{b:a}C_{in}$. The ratio of free concentration in the respiratory tract tissues to that in the liver for a given exposure concentration is:

$$\frac{C_{RT}}{C_{LI}} = 1 + Cl_{li} \left(\frac{1}{\dot{Q}_{li}} + \frac{H_{b:a}}{\dot{V}_a} \right) \qquad (4)$$

Inversely, in the low-dose region where the system is essentially linear the ratio of tissue concentrations can also be taken as the ratio of exposure concentrations that will produce the same free concentration in the liver and nasal tissue. This ratio has two terms; the first term represents the condition in which there is no metabolism and second term is associated with the metabolism of the compound in the liver.

RESULTS

Model Validation

The analysis presented in this paper is based on a PBPK model that extends previously published and validated models for styrene and chloroform to include a more detailed description of the respiratory tract. These published models have been validated for systemic blood concentrations of styrene and chloroform (Corley et al., 1990; Ramsey & Andersen, 1984), but not in the respiratory tract compartments. Respiratory tract extraction of inhaled vapors has been used previously to validate inhalation models (Bogdanffy et al., 1999b). Uptake of styrene in the nasal cavity of anesthetized CD mouse has been measured at multiple exposure concentrations (Morris, 2000). Nasal uptake measured at inspired concentrations of approximately 5, 10, 25, 50, 100, and 200 ppm at a flow rate of 12 ml/min in the mouse show a steady decrease in extraction with increasing concentration. The model accurately predicts the experimentally observed nonlinear behavior in nasal extraction as a function of inhaled concentration in mouse (Figure 2). Since nasal uptake is a function of the same processes that govern the

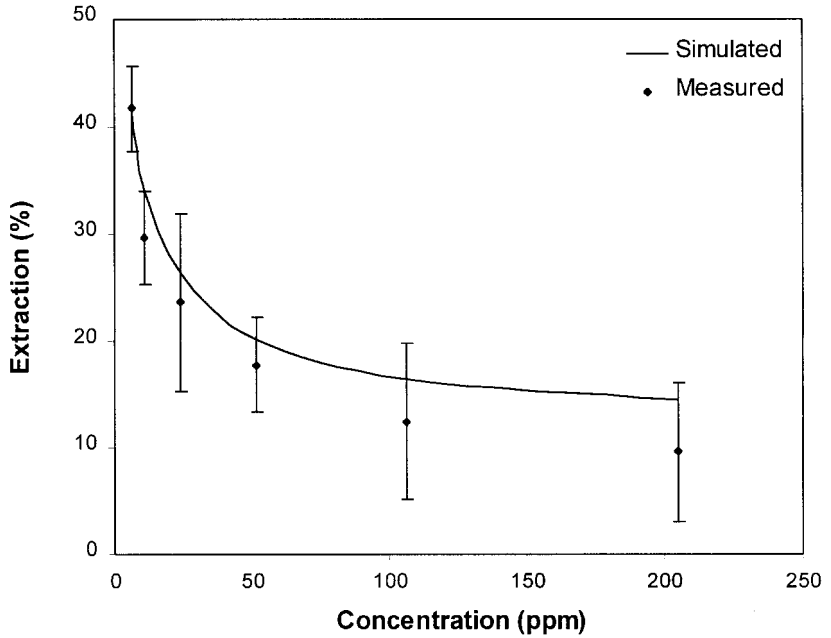

FIGURE 2. Model fit to measured extraction of styrene in mice nasal cavity for a wide range of inhaled ST concentrations (Morris, 2000). The error bars on the data points represent standard deviations.

nasal tissue concentration, a comparison of the model-derived extraction estimates to the measured nasal vapor uptake provides an indirect verification to the upper respiratory tract ST concentration. Similar extraction experiments of chloroform in the nasal cavity of rats are not available. Hence, model-predicted dose metrics for chloroform in the nasal cavity compartment cannot be validated at present.

Nasal Versus Hepatic Exposure–Dose Relationship

To study the possibility that the differential response between the nasal and hepatic tissue is due to pharmacokinetic differences in delivered dose, the PBPK model was used to simulate the amount of chloroform metabolized in the rat and styrene metabolized in the mouse, per unit tissue volume, in the nasal cavity and liver compartments for the range of exposure concentrations used in the subchronic studies (Figure 3). Model simulations were conducted until steady-state concentrations were achieved in all the tissue compartments. Model simulations predict that the exposure–dose curves for the amount of parent compound metabolized in the nasal cavity and liver tissues differ markedly, with the nasal cavity compartment showing a steeper exposure–dose relationship compared to the hepatic compartment. Both these curves indicate that the half-maximal saturation of nasal cavity metabolism occurs at a much lower inhaled concentration than does half-maximal saturation in the liver. If the amount metabolized per unit tis-

sue volume is an appropriate dose surrogate for the toxic response, these curves imply that the effective dose for a 50% response (ED50) would occur at a lower exposure concentration in the nasal tissue compared to hepatic tissue. For equivalent sensitivity of tissues in the respiratory tract and other systemic organs to metabolites of inhaled compounds, this differ-

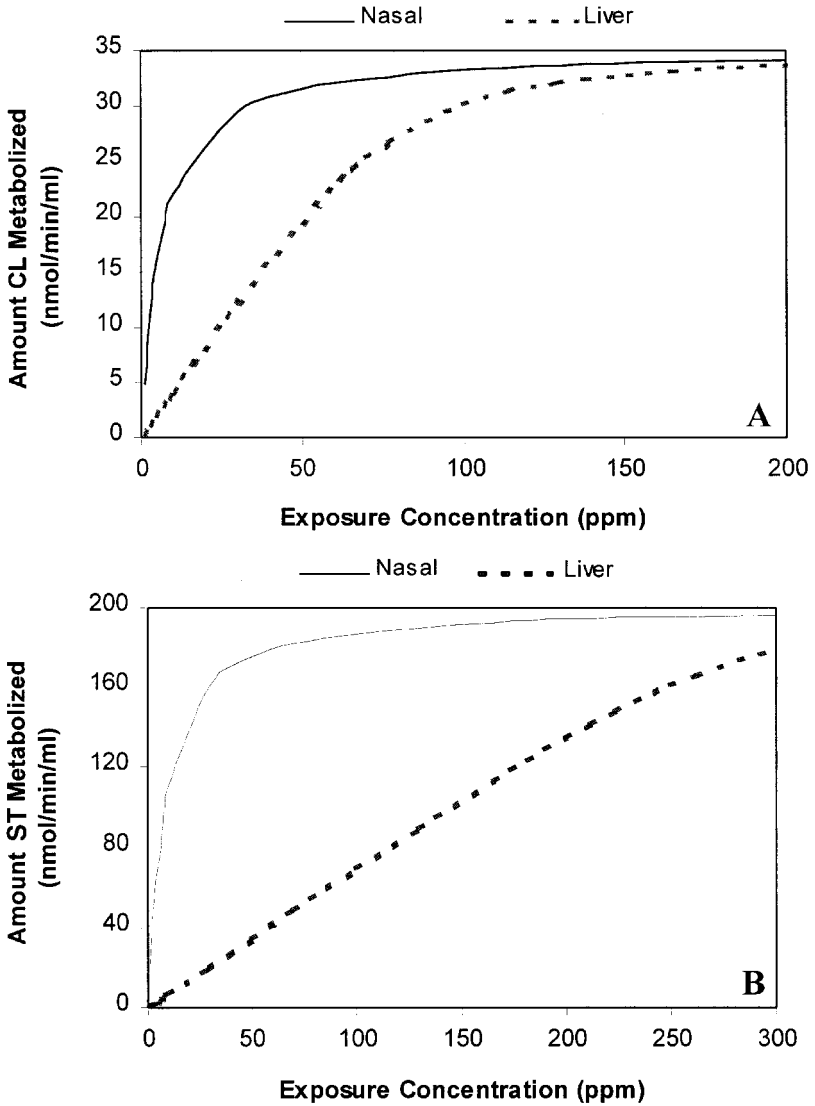

FIGURE 3. Model simulation of (A) amount of chloroform metabolized per unit tissue volume in the nasal cavity and liver in rat and (B) amount of styrene metabolized per unit tissue volume in the nasal cavity and liver in mice. All model simulations were conducted assuming a unidirectional flow in the respiratory compartments.

ence in exposure–dose curves between the portal of entry and systemic tissues could provide a PK explanation as to why nasal toxicity is present at much lower concentrations than hepatic toxicity for compounds such as styrene (Cruzan et al., 1997) and chloroform (Templin et al., 1996). Model-based predictions indicate a similar trend in the exposure–dose relationship between the conducting airways and liver tissue for inhaled volatiles (simulations not shown). This result corresponds to experimental data on the differential sensitivity of Clara-cell-rich regions of the conducting airways to toxicity from inhaled styrene at exposure concentrations lower than compared to that of the liver effects (Cruzan et al., 2001).

The issue of differential rates of metabolism for inhaled volatiles relates directly to the mode of delivery of the parent compound at the portal of entry (i.e., respiratory-tract tissues) as opposed to other systemic organs. Inhaled air directly delivers the compound to the respiratory tract tissue, whereas the arterial blood delivers the parent chemical to all the systemic organs. Model simulations were conducted for free concentration in equilibrium with blood (i.e., tissue concentration divided by the tissue:blood partition coefficient) of chloroform in the rat and free concentration of styrene in mice in the nasal and liver tissues for the range of exposure concentrations used in the corresponding subchronic studies (Figure 4). In rats, the free liver concentrations of chloroform is lower than the nasal tissue values for the tested exposure concentrations. Similarly, in mice, model simulations predict a much lower free concentration of styrene in the liver compared to the nasal cavity, which is in direct equilibrium with the exposure concentration in the air phase. Free concentrations of styrene and chloroform in the nasal tissue increase linearly with exposure (direct equilibration with air) compared to a hockey-stick type behavior in the hepatic tissue (concentrations regulated by equilibration and systemic clearance due to liver metabolism). The shallow slope for free concentration in the liver at low exposure concentrations is due to the efficient metabolic clearance of the parent chemical in the liver (Andersen, 1981). At higher exposures the metabolism in the liver is saturated, resulting in a steeper increase in hepatic free concentration.

Sensitivity analysis is another tool to understand the relationship between model parameters and model-derived dose metrics. Sensitivity analysis was conducted by measuring the change in the tissue dose metric for a prespecified change in a particular model parameter when all the other parameters are held fixed. The sensitivity coefficients represent the percent change in the model derived dose metric for a 1% change in the listed parameter. A sensitivity coefficient of 1 indicates that there is a one-to-one correlation between change in the parameter and model output. A positive value for the sensitivity coefficient indicates that the dose metric and the corresponding model parameter are directly correlated, and a negative value indicates they are inversely correlated.

The sensitivity coefficients for free tissue concentration of xenobiotics described in Table 5 were calculated at an exposure concentration of 10

TABLE 5. Sensitivity Analysis in Mice for Inhaled Styrene and in Rats for Inhaled Chloroform at the Exposure Concentration of 10 ppm for Two Different Dose Metrics (Blood Concentration and Nasal Cavity Tissue Concentration)

	Rat exposed to chloroform		Mice exposed to styrene	
Parameter	[Tissue CL]	[Blood CL]	[Tissue ST]	[Blood ST]
Cardiac output	–0.03	–0.52	–0.04	–0.7
Blood flow rate to liver	—	–0.52	—	–0.7
Blood flow rate to nasal cavity	—	—	—	—
Minute ventilation	0.064	1.0	–0.14	1.0
Tissue:air partition coefficient	0.9	0.85	—	
Composite mass transfer coefficient	0.06	—	0.12	—
Hepatic metabolic capacity	—	–0.3	—	–0.19
Nasal cavity metabolic capacity	–0.06	—	–0.2	—

Note. Sensitivity coefficients lesser than or equal to 0.01% in magnitude are considered negligible and are not reported in this table.

ppm in rats exposed to chloroform and mice exposed to styrene for two different dose metrics: steady-state blood concentration and nasal cavity tissue concentration of the inhaled volatile. Paralleling the behavior shown in Figure 4, sensitivity analysis shows that the nasal cavity tissue concentration is relatively insensitive to blood perfusion rate to the nasal cavity or other systemic parameters, including hepatic metabolism, hepatic blood flow rate, cardiac output, and minute ventilation. The nasal cavity tissue concentration is sensitive only to the tissue:air partition coefficient which essentially determines the tissue concentration for a given air-phase concentration of the inhaled volatile at equilibrium. On the other hand, arterial concentration of the inhaled volatile is sensitive to the flow parameters such as the cardiac output, hepatic blood perfusion rate, and minute ventilation, since the model behaves as a flow-limited system at low exposure concentrations.

Effect of Model Structure on Tissue Dose Metrics

Several PBPK models for inhaled vapors with effects in the deeper lung tissues have been developed. Most of these models have been structured based on a PBPK model developed for styrene (Ramsey & Andersen, 1984), leading to applications with methylene chloride (Andersen et al., 1987), chloroform (Corley et al., 1990), and trichloroethylene (Clewell et al., 2000). In all these models the inhaled volatiles are equilibrated with blood in the gas-exchange region and delivered to systemic organs and to tissues in the respiratory tract. This formulation is distinctly different from placement of the respiratory-tract tissues in intimate contact with the airways. To evaluate the effect of model structure in the respiratory-tract compartment on the corresponding tissue dose metrics, several modifications were implemented and results compared to the original model simulations.

First, the respiratory-tract tissue compartmentalization was simplified from a two-layered architecture with distinct epithelial cell layer and sub-

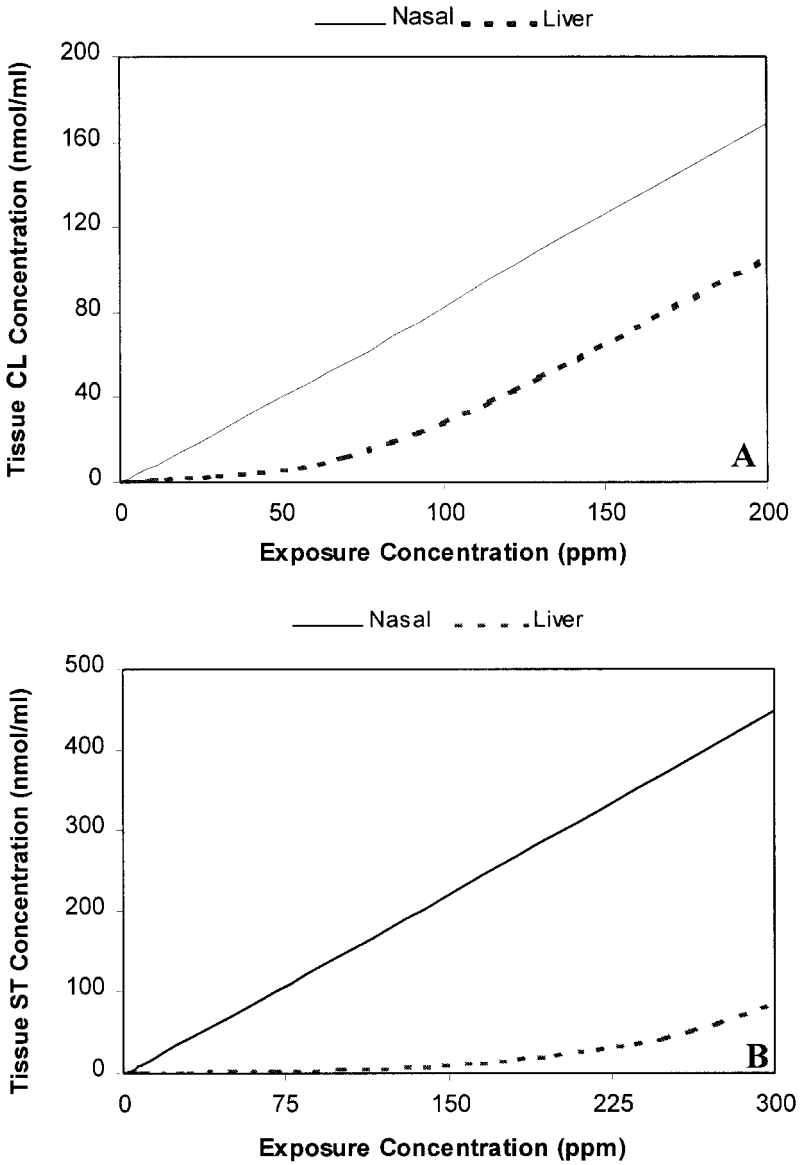

FIGURE 4. Model simulation of (A) free tissue concentration of chloroform in the nasal cavity and liver in rats and (B) free tissue concentration of styrene in the nasal cavity and liver in mice. All model simulations were conducted assuming a unidirectional flow in the respiratory compartments. Free tissue concentration is taken to be the tissue concentration divided by the tissue:blood partition coefficient.

mucosal layer to a single well-perfused tissue layer. As shown in Figure 5, parent chemical concentrations in the "lumped" single layer tissue structure are very similar to those predicted using a two-layered tissue structure. In the second modification, direct air-phase delivery of parent chemical into the anterior respiratory tract tissue compartments was eliminated and the

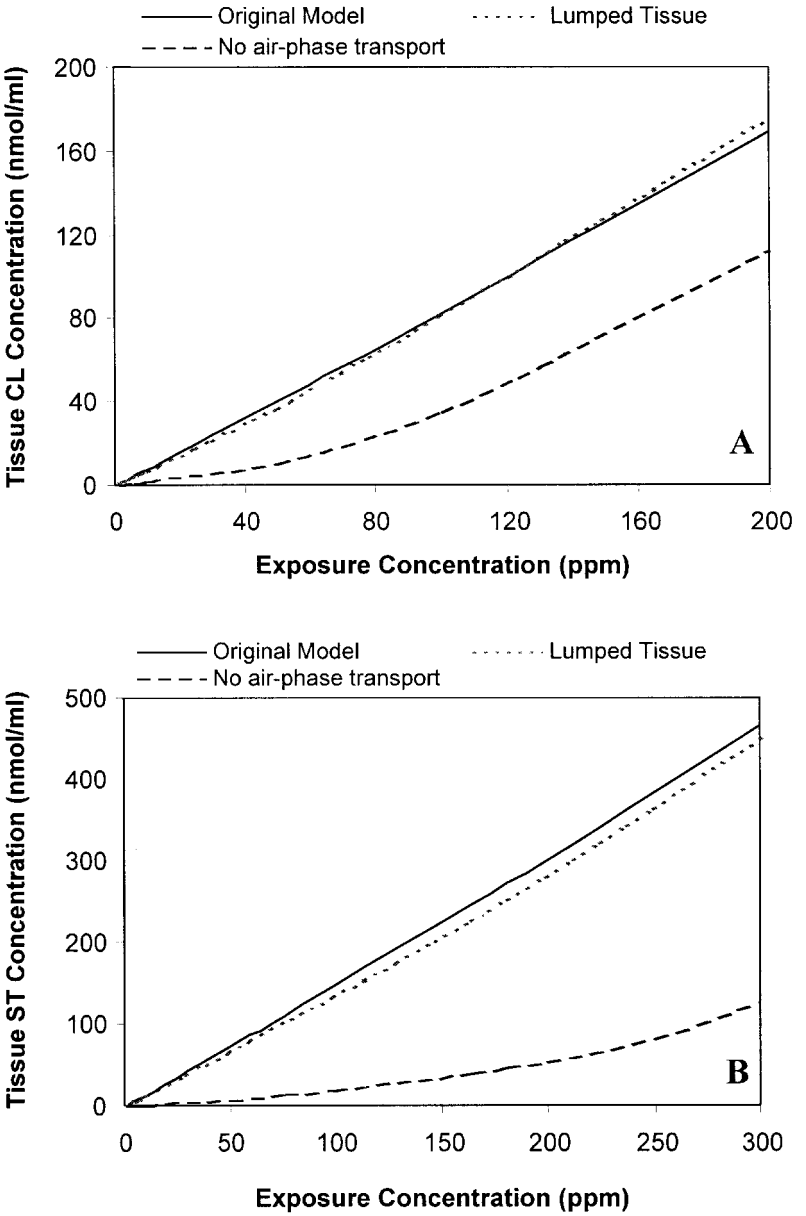

FIGURE 5. Model simulation of free tissue concentration of (A) chloroform in rats and (B) styrene in mice in the nasal cavity compartment, using the two-layer tissue structure (original model), one-layer tissue structure (lumped tissue), and tissue with only blood perfusion (no air-phase delivery). All model simulations were conducted assuming a unidirectional flow in the respiratory compartments. Free tissue concentration is taken to be the tissue concentration divided by the tissue:blood partition coefficient.

tissues were allowed to equilibrate only with the arterial blood perfusing these compartments, as in previous models for chloroform and styrene. As shown in Figure 5, elimination of the air-phase delivery results in a significantly lower prediction of the tissue concentration in the respiratory tract (Figure 5). This behavior arises because the concentration gradient of the parent chemical during inhalation at lower concentrations is from the respiratory-tract tissue into the arterial blood and not vice versa. Hence, at concentrations below those that saturate metabolism in liver, the arterial blood circulation is a clearance pathway, not a delivery pathway, for the nasal cavity and conducting airways for volatile compounds.

Theoretical Analysis on ED50 in Nasal and Liver Tissue

For styrene in mouse, the ratio of free concentrations in the nasal cavity to liver based on the theoretical calculation is about 43, while the corresponding PBPK model-predicted ratio at a low exposure concentration of 10 ppm is approximately 37. With chloroform in the rat, the ratio of nasal to liver free concentration based on the theoretical calculations is about 16, while the corresponding PBPK model-predicted ratio at a low exposure concentration of 10 ppm is approximately 13. Due to the simplifying assumptions made in the derivation of the analytical expression, it is valid only at low dose and serves as a first approximation to model-derived free concentration ratios.

The usefulness of the closed-form analytical expression is that it can also be used to compute the ratio of exposure concentrations required to produce a given free concentration in the nasal and hepatic tissues. For a free concentration equal to the K_m for metabolism, the amount metabolized per unit tissue volume is half the maximum amount metabolized at saturation. If amount metabolized per unit volume of the target tissues is a surrogate for the toxic response in the corresponding tissue, the analytical expression can be used to compute the ratio of the ED50 in the liver to that in the nasal tissue. With chloroform, which has a blood:air partition coefficient of 21 (Gargas et al., 1989), this ratio is about 16. In other words, equivalent tissue concentrations are reached in the nose at exposures predicted to be 16 times lower than those required to achieve the same concentration in the liver. In fact, nasal effects are seen at 2 ppm, while effects in other organs such as the kidney are noted at 30 ppm and above (Templin et al., 1996). For styrene, which has a blood:air partition coefficient of 40 in mice (Gargas et al., 1989), the ratio of the ED50 in the liver to that in the nasal tissue based on the theoretical analysis is about 30. The 50% response for nasal lesions in the mice is observed at about 50 ppm, while the same for hepatic lesions is observed at concentrations higher than 150 ppm (Cruzan et al., 1997), suggestive of a nasal to liver ED50 ratio of 3. On the other hand, chronic inhalation exposure to styrene in mice elicits a 100% nonneoplastic response at the lowest exposure concentration of 20 ppm but no statistically significant increase in hepatic lesions even at the highest exposure concen-

tration of 160 ppm (Cruzan et al., 2001). The lack of exact correspondence between the theoretical predictions and experimental bioassay data in mice exposed to styrene is probably due to the simplifying assumptions made in driving these analytical formulations, or it may be due to insufficient dose-response data for the nonneoplastic lesions, or it could indicate that other pharmacodynamic factors impact the chemical-induced toxic response.

DISCUSSION

Respiratory-Tract Refinements

The application of PBPK models to a variety of inhaled compounds has burgeoned in recent years. Initial applications with methylene chloride introduced metabolism in liver and lungs since these were target tissues for cancer in exposed mice. Metabolism in these tissues related to circulating toxicant concentrations rather than luminal concentrations in the airways. Models for trichloroethylene (TCE), developed for recent risk assessments for this compound, have also relied on blood delivery of TCE as the determinant of tissue metabolism for lung tumors/toxicity observed in mice with this compound (Clewell et al., 2000). In contrast, PBPK models for vinyl acetate, acrylic acid, and methyl methacrylate have focused on nasal uptake and metabolism of these compounds (Andersen et al., 1999; Bogdanffy et al., 1999a; Frederick et al., 1998). The dominant process that delivered compound to epithelial tissues in the nose in these descriptions was airflow. By developing a more complete description of the regions of the lungs, it now has become possible to assess the role of airway equilibration in controlling dose response curves for metabolism in tissues all along the airways in the respiratory tract. Our analysis shows the importance of airway equilibration for soluble, well-metabolized volatile compounds and the significant difference in the shape of curves for rate of metabolism versus inhaled concentrations in airway epithelial tissues compared to the liver. The predictions of this more complete lung model are consistent with observations of nasal/lung toxicity and toxicity of these compounds in systemic tissues. For models that assess tissue dosimetry in the respiratory tract, it will be necessary to include airway equilibration to make accurate estimates of delivered dose in these tissues. Importantly, this consideration is equally valid for compounds with high respiratory tract extraction, such as vinyl acetate (Bogdanffy et al., 1999a), or for compounds with lower extraction, such as styrene or chloroform (Morris, 2000).

Pharmacokinetic Research and Risk Assessment

The original methylene chloride and TCE models provided estimates of tissue dose for tracheobronchial effects using a structure without airway equilibration. These respiratory-tract dose estimates have been employed in several risk assessments. The misspecification in the earlier models could potentially affect risk calculations and especially in regard to the expected

differences between the test experimental animals and humans. Since model-based internal dose calculations in animal are typically conducted at high exposure concentrations and those in humans are calculated at low exposures, risk, related to the ratio of doses for humans and test animals, can potentially be altered by use of a more realistic lung equilibrium model. Recalculation of risk assessment results from application of the blood delivery model, using a more realistic lung equilibration model, should be pursued to establish the difference between the two approaches for chemicals such as TCE and methylene chloride.

The models developed here for metabolism of compounds in tissues of the respiratory tract were designed to support a more complete risk assessment with styrene in relation to lung tumors and nasal tissue toxicity (Sarangapani et al., 2001). In developing and applying the models, previous differences in dose-response relationships for inhalation toxicity in lung and liver/kidney became clarified for styrene and for other volatile compounds, such as chloroform, by elaborating the description of the respiratory tract. In the future, the elaboration of the lung to include air-phase equilibration for metabolized vapors is likely to be regarded as an essential characteristic to capture in these PBPK models. This change in perception is also likely to occur with other model characteristics in the future. Pharmacokinetics, just as with studies of modes of action or metabolism, represents both applications of mature model structures to specific compounds and research applications to assess the adequacy of present model structures. In another recent example, the pharmacokinetic model for octamethylcyclotetrasiloxan e (D4), a highly lipophilic volatile compound with very low blood:air partitioning, required consideration of slowly reversible lipid binding in blood to account for time-course data on both the blood and exhaled air concentrations of this compound (Andersen et al., 2001). This model elaboration is likely to be necessary with other compounds with similar lipid and blood solubility characteristics. Research needs for PK modeling will be repeatedly uncovered by data that do not behave as expected compared to models in current use, as with D4, and when older model structures are applied to new questions, as with the comparison of nasal and liver toxicity for chloroform or styrene. The continued development of PBPK methods in risk assessment must recognize that PBPK model building evolves in direct relation to the breadth of compounds, quality of data, and problems that are evaluated.

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