Physiologically based Pharmacokinetic Modeling of Chloroethane Disposition in Mice, Rats, and Women

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Chloroethane was observed in a chronic cancer bioassay to be a mouse-specific uterine carcinogen at a single high inhaled concentration (15,000 ppm). Although high incidence occurred in the female mouse (86%), no uterine tumor increases were observed in female rats. Chloroethane is a weak alkylating agent and has low acute toxicity. No genotoxicity potential has been observed below 40,000 ppm. Chloroethane is eliminated from the body by pulmonary exhalation and metabolically by oxidation via cytochrome P-450 (likely producing acetaldehyde) and conjugation with glutathione (GSH). The mode of action for the mouse-specific uterine tumors is not definitively known and could involve parent chemical and/or metabolite(s). A physiologically based pharmacokinetic (PBPK) model for chloroethane disposition in the rat was developed previously, but no such models have been described for mice or humans. For the work reported here, the existing PBPK model for chloroethane in rats was expanded and refined, and PBPK models for chloroethane disposition in mice and humans were developed to allow species comparisons of internal dosimetry and for possible insights into the carcinogenic and genotoxic modes of action. The amounts metabolized via glutathione-S-transferase (GST) versus cytochrome P-450, and the total amount of chloroethane absorbed, were most consistent with the observations made concerning uterine tumors, with amounts metabolized via GST providing the larger quantitative difference between the two rodent species. Choice of the most relevant dose metric for risk assessments involving uterine tumors in mice will require pharmacodynamic considerations in the mode of action in addition to the pharmacokinetic differences reported here.

Key Words: physiologically based pharmacokinetic model; PBPK model; chloroethane; P450; GST; glutathione.
Physiologically based pharmacokinetic (PBPK) models of chloroethane disposition in mice, rats and humans are useful tools for evaluating species differences in internal dose metrics of chloroethane and/or its metabolites under bioassay conditions. As indicated above, evaluation of these internal dose-response relationships can aid in evaluating hypotheses concerning potential modes of carcinogenic action. Although a dosimetry model for rats has been described (Gargas et al., 1990), these evaluations have been hampered in the past by the lack of PBPK models for chloroethane dosimetry in mice and humans. Therefore, a second and related goal of the work reported here was to develop or refine PBPK models of chloroethane disposition in female mice, female rats and women and predict internal dose metrics for comparison between these three species. We consider the models developed here as suitable for applications in assessing risk (cancer and noncancer) in humans exposed to chloroethane gas.

METHODS AND MODEL DEVELOPMENT

Animals and Chemicals

Chloroethane was purchased from Aldrich (catalog # 295110-100G). Female B6C3F1 mice (B6C3F1/CrlBR) were from Charles River Laboratory, Inc. (Raleigh, NC), a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. Body weights ranged from 19 to 23 g prior to gas uptake exposures. At the time of experimentation the mice were approximately 7–9 weeks of age. The female mice were housed singly in stainless steel, wire-mesh cages suspended above cage boards. Animal rooms were maintained at a temperature of 18°C–26°C (targeted to 22°C–24°C) and a relative humidity of 30–70% (targeted to 40–60%). Animal rooms were illuminated with fluorescent lights on a 12-h light/dark cycle that was assumed to be consistent with the light/dark cycle used for the NTP inhalation bioassay (NTP, 1989). All animals were provided tap water and fed PMI Nutrition International, LLC Certified Rodent LabDiet 5002 ad libitum. Feed and water were withheld during gas uptake exposures. Animals were sacrificed by CO2 inhalation followed by exsanguinations.

Partition Coefficients and Gas Uptake

Mouse blood was collected by exsanguination of the heart using an appropriately sized syringe and needle pretreated with sodium heparin to prevent clotting. Mouse blood-to-air, buffer-to-air and boved liver microsome-to-air partition coefficients (PCs) were determined using a previously described procedure (Gargas et al., 1989).

Gas uptake exposures were conducted in female B6C3F1 mice using a system similar to the one described by Evans and associates (Evans et al., 1994). Three mice were used for each exposure using nominal starting concentrations of between 100 and 3000 ppm. Oxygen in the chamber was monitored continuously using a Biosystems model 3100R O2 gas monitor (Biosystems Inc., Middletown, CT) and maintained between 19 and 21%. Carbon dioxide and ammonia were removed by circulating the system air through soda lime (Fluka, Sigma-Aldrich Corp., St Louis, MO) and sodium citrate (Aldrich, Sigma-Aldrich Corp., respectively. Chamber chloroethane concentrations were monitored starting 10 min after vapor introduction and then every 10 min to the end of exposure.

Human Kinetic Constants of Metabolism

The human kinetic constants for metabolism were determined for the oxidative pathway of chloroethane metabolism (VMAXOxyv, and KMox) using human microsomal preparations and fitting of in vitro gas uptake data (see Supplementary Data for details). The rodent VMAXOxy, that is, the maximum rate for GST conjugation of chloroethane, was estimated from experimental data and that for humans was determined using a parallelogram approach. The KMox was assumed to be constant among species (see Supplementary Data for details). The human GST most likely responsible for metabolizing chloroethane is the theta class GST (GSTT1-1), which has been shown to metabolize methyl chloride, a structural analog of chloroethane (Thier et al., 1998). GSTT1-1 is polymorphic in humans, exhibiting three distinct groups of individuals (Pemble et al., 1994; Thier et al., 1998) that have been classified as high conjugators (HCs), low conjugators (LCs), or nonconjugators (NCs), representing individuals that are homozygous, heterozygous, or null for the allele. This polymorphism was accounted for in the human modeling performed here, with the LC having about one-half the activity of the HC (as was found for methyl chloride by Thier et al., 1998) and the NC having no activity.

Analytical

Chloroethane as a pure gas (10⁶ ppm) was transferred to an empty Tedlar bag (~1 l, SKC Inc., Eighty Four, PA). The dilutions in additional Tedlar bags containing known volumes of room air were used for calibration or exposure purposes. Gas transfers were made with gas tight syringes (Hamilton Company, Reno, NV and SGE Incorporated, Austin, TX).

Gas chromatography (GC) was used for quantitative analysis of chloroethane. The GC equipment was the Agilent 6890 GC with detection by flame ionization. Chromatography used a JW model 125-5032 DB5 megabore 0.53-mm-internal diameter capillary column (Agilent, Wilmington, DE). Sample introduction from the in vivo gas uptake sampling was by automated pneumatic sampling valve (250 µl, Agilent). Sample introduction for the PCs determinations was performed using a robotic x-y-z programmable multipurpose sampler (MPS2, Gerstel US, Baltimore, MD). The injection volume was 100 µl. Calibrations were performed over concentrations ranging from 5 to 6000 ppm.

Model Development

Model structure. The structure of the expanded PBPK model is depicted in Figure 1. The Gargas et al. (1990) model for rats was used as the starting point for all three species. Compartmental for the refined model were included as necessary for mathematical description related to potential model(s) of action (including uterus and adrenal glands), storage functions (slowly perfused tissues and depot fat), major site of oxidative metabolism and GST conjugation (liver), secondary sites of GST metabolism with available GSH depletion data (kidney, brain, and gonads [ovary or testes]), and for mass/blood flow balance (other rapidly perfused tissues). The main difference from the model structure employed by Gargas et al. (1990) was that the uterus, adrenal glands, kidney, brain, and ovary/testes were described as discrete tissues rather than as part of the “other rapidly perfused tissues” compartment.

Oxidative metabolism of chloroethane by cytochrome P450 was described as being saturable with respect to chloroethane concentration, as demonstrated by Gargas et al. (1990). Conjugation of GST with chloroethane was described in this current work as saturable with respect to both chloroethane concentrations and GST concentrations, respectively. Tissue GST concentrations were described as resulting from a balance of zero-order input (synthesis or delivery) and first-order loss (cellular uses, degradation, or export). A complete mathematical description of the model is provided in the Supplementary Data.

Physiological parameters. Model parameters were summarized in Table 1 for certain parameters with a complete listing of model parameters in the Supplementary Data. To the extent possible, physiological parameters (body and organ weights, alveolar ventilation, and blood flows) were taken from the existing chloroethane model (Gargas et al., 1990) and an existing literature review (Brown et al., 1997). Additionally, a published PBPK model for estradiol in the rat and human (Plowchalk and Teeguarden, 2002) was also used as a source for physiological parameter values for reproductive tissues not surveyed by Brown et al. (1997).

Partition coefficients. In vitro rat tissue:air PCs as measured by Gargas et al. (1989) are used for the rat model. The rat PC for liver was used for...
Several data sets were used in parameter fitting and model validation, as described in Results. The maximum rate of rat oxidative metabolism of chloroethane (VMAXoC) and the enzyme affinity for this reaction (KMo) were the values derived by Gargas et al. (1990). VMAXoC and KMo for the mouse were determined in this current work by optimization of the model fit to closed-chamber gas uptake data as per Gargas et al. (1990). VMAXoC and KMo for humans were determined by optimization of fit to in vitro vial headspace disappearance of chloroethane due to human female liver microsomal metabolism. The in vitro metabolic rate was scaled to an estimated whole-body rate based on estimated liver weight and microsomal protein yield. Details on the determination of the human oxidative rate parameters are provided in the Supplementary Data.

The maximum rate of enzymatic chloroethane-GSH conjugation in rat and mouse liver (VMAXgC) was determined by fit to liver GSH depletion (Pottenger et al., 1992) at a high chloroethane concentration (approximately 15,000 ppm inhaled). The affinity of chloroethane for GST for this reaction (KMG) was calculated based on equivalence at low concentrations between the first-order rate for rat liver determined by Gargas et al. (1990), and the saturable rate equations used herein (Supplemental Materials). For GSH conjugation in extrahepatic tissues, the enzyme affinity was assumed to be the same as in liver, and the capacity in rats and mice was calculated as a fraction of the liver capacity (optimized by fit to GSH depletion data from Pottenger et al., 1992).

VMAXgC for humans was estimated using the parallellogram (corollary) approach, assuming similarities in in vitro metabolic activity ratios (human vs. rodent) between chloroethane and the structurally similar compound chloromethane. Extrahepatic capacities for humans were likewise estimated by the parallellogram approach. Details are provided in the Supplemental Materials.

Software and Algorithms

All simulations and parameter fitting were conducted using ACSL Sim and ACSL Math, Version 11.4 (Aegis Technologies, Huntsville, AL) on a Dell Optiplex GX260 computer with a Pentium 4 processor. The Gear algorithm was used for integration of double precision variables. Parameter fitting was performed using the relative error model (variance is assumed to be proportional to the measured value across the range of measured values, or heteroscedasticity = 2) and the Nelder-Mead algorithm. The fitting criterion was maximization of the log likelihood function. Starting values for parameter fitting in ACSL Math were determined from parameter estimates derived by visual best-fit in ACSL Sim. Model equations, in mathematical form, are provided in the Supplementary Data. Model code is available from the authors upon request.

RESULTS

Parameter Fitting and Validation

Rat Parameter Fitting: GSH Depletion Data

GSH-conjugation rates for the liver, kidney, brain, ovary, adrenal gland, and uterus were determined using the tissue GSH concentrations measured by Pottenger et al. (1992). We observed that the initial values selected for the GSH turnover rate in ovary, adrenal gland and uterus, initially estimated to be similar to the turnover rates observed in a representative richly perfused tissue (small intestine) by Potter and Tran (1993), were not adequate to describe the quick replenishment of GSH in these tissues after cessation of chloroethane exposure. Therefore tissue-specific GSH turnover rates in the rat ovary, adrenal gland and uterus were also determined using ACSL Math optimization of model predictions of measured tissue.

Biochemical parameters. Default initial rat and mouse tissue concentrations of GSH were taken from Pottenger et al. (1992). These default values were used in modeling in the absence of experiment-specific control GSH data; when such data were available, the experiment-specific control values were used. First-order GSH loss rates for specified or similar tissues were taken from Potter and Tran (1993) for use as initial estimates.
**TABLE 1**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Symbol</th>
<th>Rat</th>
<th>Mouse</th>
<th>Human</th>
<th>Units</th>
<th>Source/comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood:air PC</td>
<td>PB</td>
<td>5.5</td>
<td>5.1</td>
<td>2.69</td>
<td>(None)</td>
<td>For rat, see text/this paper/Gargas et al. (1989)</td>
</tr>
<tr>
<td>Initial concentration of GSH in the liver</td>
<td>GSHL0</td>
<td>5.78</td>
<td>5.45</td>
<td>5.63</td>
<td>mM</td>
<td>Pottenger et al. (1992) (rat and mouse default values; reported experimental values used when available); Sweeney et al. (2003) (human)</td>
</tr>
<tr>
<td>Initial concentration of GSH in the kidneys</td>
<td>GSHK0</td>
<td>3.8</td>
<td>3.05</td>
<td>1.39</td>
<td>mM</td>
<td>Pottenger et al. (1992) (rat and mouse default values; reported experimental values used when available); Sweeney et al. (2003) (human)</td>
</tr>
<tr>
<td>Initial concentration of GSH in the brain</td>
<td>GSHN0</td>
<td>1.59</td>
<td>1.59</td>
<td>2.99</td>
<td>mM</td>
<td>Pottenger et al. (1992) (rat and mouse default values; reported experimental values used when available); Sweeney et al. (2003) (human)</td>
</tr>
<tr>
<td>Initial concentration of GSH in the ovaries (gonads)</td>
<td>GSHG0 (female)</td>
<td>2.8</td>
<td>1.94</td>
<td>2.4</td>
<td>mM</td>
<td>Pottenger et al. (1992) (rat and mouse default values; reported experimental values used when available); average of rat and mouse values (human)</td>
</tr>
<tr>
<td>Initial concentration of GSH in the uterus</td>
<td>GSHU0</td>
<td>1.18</td>
<td>1.65</td>
<td>1.5</td>
<td>mM</td>
<td>Pottenger et al. (1992) (rat and mouse default values; reported experimental values used when available); Serviddio et al. (2002) (human)</td>
</tr>
<tr>
<td>Initial concentration of GSH in the adrenal glands</td>
<td>GSHD0</td>
<td>2.62</td>
<td>1.85</td>
<td>1.8</td>
<td>mM</td>
<td>Pottenger et al. (1992) (rat and mouse default values; reported experimental values used when available)</td>
</tr>
<tr>
<td>First-order loss rate of GSH from liver</td>
<td>KDGSHL</td>
<td>0.142</td>
<td>0.41</td>
<td>0.28</td>
<td>/h</td>
<td>Potter and Tran (1993) (rat); fit to GSH depletion data (Pottenger et al., 1992) (mouse)</td>
</tr>
<tr>
<td>First-order loss rate of GSH from kidney</td>
<td>KDGSHK</td>
<td>0.55</td>
<td>0.36</td>
<td>0.46</td>
<td>/h</td>
<td>Potter and Tran (1993)—rat; fit to GSH depletion data (Pottenger et al., 1992) (mouse)</td>
</tr>
<tr>
<td>First-order loss rate of GSH from brain</td>
<td>KDGSHN</td>
<td>0.044</td>
<td>0.042</td>
<td>0.043</td>
<td>/h</td>
<td>Potter and Tran (1993)—rat small intestine (representative RPT); fit to GSH depletion data (Pottenger et al., 1992) (mouse)</td>
</tr>
<tr>
<td>First-order loss rate of GSH from ovaries (gonads)</td>
<td>KDGSHG (female)</td>
<td>0.15</td>
<td>0.22</td>
<td>0.19</td>
<td>/h</td>
<td>Fit to GSH depletion data (Pottenger et al., 1992) (rat and mouse)</td>
</tr>
<tr>
<td>First-order loss rate of GSH from uterus</td>
<td>KDGSHU</td>
<td>0.12</td>
<td>0.45</td>
<td>0.29</td>
<td>/h</td>
<td>Fit to GSH depletion data (Pottenger et al., 1992) (rat and mouse)</td>
</tr>
<tr>
<td>First-order loss rate of GSH from adrenal glands</td>
<td>KDGSHD</td>
<td>0.31</td>
<td>0.73</td>
<td>0.52</td>
<td>/h</td>
<td>Fit to GSH depletion data (Pottenger et al., 1992) (rat and mouse)</td>
</tr>
<tr>
<td>Maximal rate of oxidative metabolism of chloroethane (normalized to body weight)</td>
<td>VMAXoC</td>
<td>4</td>
<td>10.9</td>
<td>25.9</td>
<td>mg/h-kg^{0.7}</td>
<td>Gargas et al. (1990) (rat); fit to gas uptake data (this manuscript) (mouse); scaled from fit to in vitro gas uptake data (this manuscript)</td>
</tr>
</tbody>
</table>
GSH concentrations. This optimization was concurrent with the optimization of GSH-conjugation capacity of the tissue. The fits of the refined model to the liver and uterus GSH depletion data are shown in Figure 2; GSH depletion plots for other tissues may be found in the Supplementary Data.

**Rat Validation**

**Closed-chamber chloroethane uptake.** The change in the description of GSH conjugation in the liver (from apparently first-order to saturable kinetics, to appropriately characterize limitations in GSH conjugation at higher concentrations of chloroethane) and the addition of GSH conjugation in extrahepatic tissues did not alter the model fits to the closed-chamber data (Gargas et al., 1990) (figure provided in Supplementary Data).

**GSH depletion.** Fedtke et al. (1994b) exposed male and female F344 rats to a nominal concentration of 15,000 ppm chloroethane or air for 6 h/day, 5 days/week and measured liver, kidney, and uterus (female only) GSH concentrations. The reported tissue GSH concentrations in the air-exposed controls were used as the initial tissue GSH concentrations for exposed animals (as no t = 0 h control values were reported).

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**TABLE 1—Continued**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Symbol</th>
<th>Rat</th>
<th>Mouse</th>
<th>Human</th>
<th>Units</th>
<th>Source/comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme affinity of chloroethane for oxidative metabolism</td>
<td>Kmo</td>
<td>0.1</td>
<td>1.69</td>
<td>1.03</td>
<td>mg/l</td>
<td>Gargas et al. (1990) (rat); fit to gas uptake data (this manuscript) (mouse), fit to in vitro gas uptake data (this manuscript) (human)</td>
</tr>
<tr>
<td>Maximal rate of GST conjugation in the liver (normalized to body weight)</td>
<td>VMAXgC</td>
<td>0.59</td>
<td>2.61</td>
<td>1.03, 0.515, 0</td>
<td>mg/h-kg(^0.7)</td>
<td>Fit to liver GSH depletion data (15,000 ppm Pottenger et al., 1992) (rat and mouse); estimated from parallelogram approach (human HC, LC, or NC) (Thier et al., 1998)</td>
</tr>
<tr>
<td>Enzyme affinity of chloroethane for GSH conjugation</td>
<td>KMg</td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>mg/l</td>
<td>Assume Gargas Kf = VmaxgC/Km (rat); assume KM constant across species (mouse, human)</td>
</tr>
<tr>
<td>Enzyme affinity of GSH for enzymatic GSH conjugation</td>
<td>KMgSH</td>
<td>0.125</td>
<td>0.125</td>
<td>0.125</td>
<td>mM</td>
<td>Ivanetich and Goold (1989)</td>
</tr>
<tr>
<td>CE-GST conjugation capacity of kidneys (fraction of liver capacity)</td>
<td>FRACGK</td>
<td>0.19</td>
<td>0.086</td>
<td>0.15</td>
<td>(None)</td>
<td>Fit to GSH depletion data (Pottenger et al., 1992) (rat and mouse); estimated from parallelogram approach (human) (Thier et al., 1998)</td>
</tr>
<tr>
<td>CE-GST conjugation capacity of brain (fraction of liver capacity)</td>
<td>FRACGN</td>
<td>0.0067</td>
<td>0.010</td>
<td>0.058</td>
<td>(None)</td>
<td>Fit to GSH depletion data (Pottenger et al., 1992) (rat and mouse); estimated from parallelogram approach (human) (Thier et al., 1998)</td>
</tr>
<tr>
<td>CE-GST conjugation capacity of ovaries (gonads) (fraction of liver capacity)</td>
<td>FRACGG (female)</td>
<td>0.011</td>
<td>0.00094</td>
<td>0.048</td>
<td>(None)</td>
<td>Fit to GSH depletion data (Pottenger et al., 1992) (rat and mouse); estimated from parallelogram approach (human) (Thier et al., 1998)</td>
</tr>
<tr>
<td>CE-GST conjugation capacity of uterus (fraction of liver capacity)</td>
<td>FRACGU</td>
<td>0.014</td>
<td>0.013</td>
<td>0.028</td>
<td>(None)</td>
<td>Fit to GSH depletion data (Pottenger et al., 1992) (rat and mouse); estimated from parallelogram approach (human) (Thier et al., 1998)</td>
</tr>
<tr>
<td>CE-GST conjugation capacity of adrenal glands (fraction of liver capacity)</td>
<td>FRACGD</td>
<td>0.0098</td>
<td>0.0022</td>
<td>0.0099</td>
<td>(None)</td>
<td>Fit to GSH depletion data (Pottenger et al., 1992) (rat and mouse); estimated from parallelogram approach (human) (Thier et al., 1998)</td>
</tr>
</tbody>
</table>
Landry et al. (1982) exposed male F344 rats to 0, 1600, 4000, or 10,000 ppm chloroethane for 6 h, and measured liver GSH concentrations 0.5 h after the end of exposure. Comparisons of measured and model-predicted values are presented in Table 2.

**Mouse Parameter Fitting: Oxidative Metabolism**

Gas uptake studies conducted using chambers containing three female B6C3F1 mice for up to 6 h were the key data set for determining rates of oxidative metabolism of chloroethane. Best-fit values of VMAXoC and KMo (see Table 1) were determined by simultaneously optimizing the fit of the model to all 12 closed-chamber runs. The fits of the model predictions to the closed-chamber gas uptake data are shown in Figure 3.

**Mouse Parameter Fitting: GSH Metabolism and Synthesis**

The key data set describing GSH depletion in chloroethane-exposed mice was reported by Pottenger et al. (1992). Both GSH-conjugation rates and tissue-specific GSH turnover rates were determined using ACSL Math (see Table 1). Based on visual inspection of the output, the fit to the measured GSH concentrations in adrenal glands and ovary did not adequately capture the replenishment of GSH during early exposure. Rather than optimize the fit to the “absolute” GSH concentrations in the adrenal glands and ovary, the model was fit to GSH concentrations as a fraction of the time-matched control level of GSH measured by Pottenger et al. (1992). The fits of the model to the GSH depletion data for the liver and the uterus are shown in Figure 4; additional tissue GSH depletion plots may be found in the Supplemental Materials. For ovary and adrenal gland, comparisons to both absolute and relative GSH concentrations are shown.

**Mouse Validation**

**GSH depletion.** Fedtke et al. (1994b) exposed male and female B6C3F1 mice to a nominal concentration of 15,000 ppm chloroethane or air for 1 week (6 h/day, 5 days/week), and measured liver, kidney, and uterus (female only) GSH concentrations. The reported tissue GSH concentrations in the air-exposed controls were used as the initial tissue GSH concentrations for exposed animals (no t = 0 h control values were reported). Landry et al. (1982) exposed male B6C3F1 mice to 0 or 4000 ppm chloroethane for 6 h, and measured liver GSH concentrations 0.5 h after the end of exposure. Pottenger et al. (1992) exposed female B6C3F1 mice to a nominal concentration of 6000 ppm chloroethane for 6 h and measured GSH at the end of the exposure. Comparisons of measured and model-predicted values are presented in Table 3. The predictions of GSH concentrations after single exposures (Landry et al., 1982; Pottenger et al., 1992) were reasonably accurate. For repeat exposures (Fedtke et al., 1994b), GSH predictions in liver and kidney were less accurate.

**Human Model Validation**

The development of the human model was described in Methods and in the Supplemental Materials. Limited validation data for humans were available (Morgan et al., 1970). In this study, human volunteers (unspecified number) wearing a nose clip deeply inhaled ~5 mg of radioactive chloroethane and held their breath for approximately 20 s. Total time for administration and breath holding was approximately 30 s. After breath holding, the subject inhaled room air and exhaled into a charcoal trap. Thereafter traps were changed at 2- to 10-min intervals for 1 h. Morgan et al. (1970) indicate that roughly 18% of inhaled chloroethane (measured as radiolabel chlorine) was exhaled (82% retained) at the end of the breath-holding period and after 1 h, 30% of the retained chloroethane (measured as radiolabel chlorine) was exhaled in breath. This scenario was approximated by simulating an exposure to 870 ppm chloroethane for 0.0083 h (30 s), because this scenario produces total intake of 5 mg chloroethane. In the...
TABLE 2
Comparison of Measured and Model-Predicted Values of Tissue GSH (Fraction of Control Concentration) in Chloroethane-Exposed F344 Rats

<table>
<thead>
<tr>
<th>Study</th>
<th>Liver GSH</th>
<th>Kidney GSH</th>
<th>Uterus GSH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Measured</td>
<td>Predicted</td>
<td>Measured</td>
</tr>
<tr>
<td>Fedtke et al. (1994b)</td>
<td>0.9 ± 0.2</td>
<td>0.67</td>
<td>0.66 ± 0.09</td>
</tr>
<tr>
<td>Females</td>
<td>14,393 ppm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fedtke et al. (1994b)</td>
<td>0.65 ± 0.09</td>
<td>0.72</td>
<td>0.75 ± 0.15</td>
</tr>
<tr>
<td>Males</td>
<td>14,090 ppm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Landry et al. (1982)</td>
<td>0.95 ± 0.06</td>
<td>0.89</td>
<td>ND</td>
</tr>
<tr>
<td>Males</td>
<td>1600 ppm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Landry et al. (1998)</td>
<td>0.88 ± 0.04</td>
<td>0.84</td>
<td>NA</td>
</tr>
<tr>
<td>Males</td>
<td>4000 ppm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Landry et al. (1998)</td>
<td>0.89 ± 0.06</td>
<td>0.82</td>
<td>NA</td>
</tr>
<tr>
<td>Males</td>
<td>10,000 ppm</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note. Estimated control (t = 0) concentrations of GSH were as follows. From Fedtke et al. (1994b) figures. Females: liver GSH = 4.2mM, kidney GSH = 4.3mM, uterus GSH = 1.2mM; males: liver GSH = 4.7mM, kidney GSH = 3.2mM. Landry et al. (1982) males: liver GSH 7.2mM. ND = not determined. NA = not applicable.

*Animals exposed 6 h/day for 5 days, with GSH measured at the end of exposure.

*Animals exposed for 6 h, with GSH measured 0.5 h after the end of exposure.

Simulations, it was predicted that of 5 mg inhaled, 1.5 mg (30%) would be exhaled (3.5 mg or 70% retained) in the first 0.0083 h (30 s). This simulation would be expected to slightly underpredict the amount of chloroethane retained after breath holding because in breath holding, some chloroethane would be swept away from the lungs by blood flow with no concomitant exhalation. Thus the model-predicted value of 70% retained (30% exhaled) is reasonably accurate and demonstrates that a significant portion of excretion is by exhalation. The model predicts that an additional 1.1 mg would be exhaled in the next hour. This prediction of 31.4% of retained chloroethane being exhaled within an hour is very close to the experimentally observed value of 30%. These simulations indicate that the PCs and estimated total metabolic rates provide reasonably accurate predictions of the overall disposition of inhaled chloroethane for human volunteers. These simulations cannot, however, provide validation of the split of total metabolism between oxidative and GST-mediated pathways.

**Evaluation of the Output of the Chloroethane Models—Species Comparisons**

Several internal measures of dose were predicted for mice, rats and humans following simulated exposures to 0–15,000 ppm chloroethane using the PBPK models described above. Blood (Fig. 5a) and uterus Fig. 5b) concentrations at the end of 6-h inhalation exposures and the amounts of chloroethane metabolized via cytochrome P-450 (Fig. 6) and via GST (Fig. 7) in a 24-h period during and following 6-h inhalation exposures were simulated. Blood concentrations of chloroethane (as well as uterus concentrations) were quite similar for the three species over the range of inhaled concentrations evaluated, with humans predicted to have somewhat lower concentrations compared with rodents. The amounts of chloroethane predicted to be metabolized via cytochrome P-450 and GST demonstrated larger differences between the species compared with blood concentrations, with mice metabolizing more through either pathway compared with rats at the same inhaled concentrations and humans metabolizing the least amount under the same exposure conditions. Nonlinear kinetics observed in the predicted metabolism curves (Figs. 6 and 7) are consistent with metabolic saturation of both cytochrome P-450 and GST, albeit at different inhaled concentrations of chloroethane. Saturation of the cytochrome P-450 pathway is predicted to be nearly complete at 200–500 ppm in rats and above 1000 ppm in mice, with humans saturating this pathway in the 1000- to 3000-ppm range (Fig. 6). Saturation of GST is nearly complete for all three species in the 6000- to 9000-ppm range of inhaled chloroethane (Fig. 7). Above concentrations where saturation is complete, the amount metabolized in 24 h (6-h exposure plus 18-h postexposure) becomes almost constant for mice and rats but is predicted to continue to increase, albeit at a slower rate, for the three classes of humans (Figs. 6 and 7). The difference among species may be due to the greater importance of postexposure metabolism for humans, who would be slower to clear chloroethane from the body by exhalation, due to a slower breathing rate per kg body weight.

A direct quantitative comparison can be made regarding the amounts metabolized via either pathway for the three species over the inhaled concentration range evaluated (1–15,000 ppm). Rats and humans were predicted to produce less metabolite via both pathways as compared with the mouse, with the human producing much less of both at the lower inhaled concentrations. When exposed under the bioassay conditions (6 h to 15,000 ppm chloroethane), the model predicted that a rat (0.25 kg) will metabolize 48.8 mg/kg-day via cytochrome P-450 and 6.8 mg/kg-day via GST, the mouse (0.025 kg) will metabolize 211 mg/kg-day via cytochrome P-450 and 6.8 mg/kg-day via GST.
P-450 and 48 mg/kg-day via GST, whereas the HC woman (58 kg) was predicted to metabolize 95.5 mg/kg-day via cytochrome P-450 and 2.5 mg/kg-day via GST. Simulations were also conducted to determine what continuous exposure concentrations (24 h/day, 7 days/week) would be necessary for women to metabolize as much chloroethane via the cytochrome P-450 or GST pathway as was predicted for rodents exposed under bioassay conditions. To achieve the fluxes predicted for rats, women would have to be exposed to greater than 340 ppm (cytochrome P-450 pathway) or 3600 ppm (GST pathway). Only women who are HCs would be able to achieve the GST flux predicted for rats. To achieve the cytochrome P-450 pathway flux predicted for mice, the species susceptible to chloroethane-induced uterine tumors, women would need to be continuously exposed to 2000 ppm chloroethane. The GST pathway fluxes predicted for mice cannot be achieved in exposed women, even HCs due to the limited GSH-conjugation capacity in women.

FIG. 3. Closed-chamber uptake of chloroethane by three female B6C3F1 mice exposed 6 h to 100, 300, or 1000 ppm (b) and 2000, 2500, or 5000 ppm (a) chloroethane. Exposure lines are identified at t = 0, at y intercept. Symbols = experimental data. Lines: model predictions. $r^2 = 98$.

FIG. 4. GSH concentrations or fraction of control GSH in tissues of chloroethane-exposed mice. (a) Liver, (b) uterus. Female B6C3F1 mice were exposed for 6 h to 15,290 ppm chloroethane. Line: best-fit model simulation. Symbols: data of Pottenger et al. (1992). $n = 8$ for liver, $n = 4$ for uterus. $r^2$: liver, 99.6; uterus, 95.

DISCUSSION

Models of chloroethane absorption, disposition, metabolism, and elimination have been developed and validated for the mouse, rat, and human. Refinements (as compared with the Gargas et al., 1990 rat model) include description of saturable conjugation of chloroethane with GSH, extrahepatic GSH conjugation, incorporation of GSH production/synthesis and degradation/export in liver and extrahepatic tissues, and the inclusion of additional discrete tissue compartments, including those believed relevant to the mode of action for cancer in the mouse. These current models performed quite well in fitting much of the available data (see further discussion below) and although certain limitations have also been identified (also see below), we view these current models as valuable tools for assessing internal doses over a wide range of inhaled chloroethane concentrations for three species of interest. These
current models are well suited for use in quantitative risk assessments for chloroethane, and can provide useful insights into hypotheses proposed for carcinogenic mode(s) of action.

Comparing the fits of model predictions to measured data is key to evaluating the performance and utility of the model as well as for identifying potential areas of improvement. The fit of the model to measured rat GSH data is generally very good (Fig. 2, Table 2, Supplementary Data), but was limited by the form of GSH submodel, which incorporates no overshoot (temporary rebound to GSH concentrations exceeding the initial concentration) and assumes an eventual return to control levels after cessation of exposure. There are also possible limitations in the data, in that sample numbers may not adequately characterize the “true” response of the animals. The fit to the closed-chamber data was not altered by the model refinement (shown in Supplementary Data), and indicated that whole-body metabolism was accurately described and that extrahepatic metabolism (added to permit simulation of extrahepatic GSH depletion) was relatively minor, with respect to whole-body chloroethane metabolism. The agreement between rates of oxidative metabolism in the rat determined by Gargas et al. (1990) and scale-up of in vitro data (Fedtke et al., 1994a) further supports the division of whole-body metabolism of chloroethane between oxidative and GSH pathways.

The fit of the model to the gas uptake data (Fig. 3) in mice was excellent. The largest deviations between the model and experimental data were at the lowest starting concentrations, where uptake (metabolism) by the mice was underpredicted by the model. A possible explanation is that extrahepatic CYP 2E1 makes a contribution to whole-body metabolism at low concentrations that is less important at higher concentrations. This phenomenon could also be explained by a GSH-conjugation compartment that might be functioning at low exposures, but is rapidly exhausted at higher concentrations and thus loses its ability to serve as a low-exposure sink. One such pool in rodents might be the red blood cell.

For single exposures in mice, the predictions of tissue GSH concentrations were very good. The model underpredicted liver and kidney GSH concentrations after repeated exposure.
In vitro data indicate that chloroethane-exposed mice have increased rates of liver microsomal chloroethane metabolism and decreased rates of liver cytosol chloroethane-GSH conjugation relative to air-exposed mice (Fedtke et al., 1994a, b). Although these changes may partially explain the differences between measured and predicted liver GSH concentrations for female mice, it cannot account for the increased amount of GSH in male mouse livers at the end of the exposure. A future model refinement might include incorporation of stimulated GSH synthesis upon repeated exposures.

Optimized parameters for oxidative metabolism in female mice (VMAXoC, KMo) were determined by best-fit to closed-chamber gas uptake data (Fig. 4). The enzyme affinity determined for female mice (1.69 mg/l) is significantly different from that determined for rats (0.1 mg/l, Gargas et al., 1990). Although this was unexpected, large differences in KM determined by gas uptake have previously been identified for another CYP 2E1 substrate, vinyl chloride (0.04 mg/l in rats, 0.28 mg/l in mice, Reitz et al., 1996). The KM values determined by gas uptake are in units of parent chemical that is in equilibrium with the venous blood leaving the metabolizing organ and have been described as representing a possible upper bound on the true binding affinity at the enzyme (Gargas et al., 1990). This could also impact the comparability of the KM values used here. The optimized in vivo Vmax for the oxidative pathway was also larger in mice than in rats (10.9 vs. 4 mg/h-kg0.7), which is consistent with in vitro findings where metabolism of chloroethane was determined to be ~2× greater in mouse microsomes than in rat microsomes (Fedtke et al., 1994a). For GSH conjugation in the liver, the Vmax values for rats and mice follow the same pattern, with an in vivo mouse:rat ratio of ~4 and in vitro ratio of ~5 (Fedtke et al., 1994b). No comparisons of KMs were possible because the Fedtke et al. in vitro determinations were conducted only at high chloroethane concentrations. The enzymatic rate of GSH conjugation in extrahepatic tissues (Table 1), estimated as a fraction of the rate in the liver via fitting GSH depletion data, appeared to be similar in rats and mice for kidney, brain, uterus, and adrenal gland (within roughly a factor of 2), but was substantially lower in mouse ovary, as compared with rat ovary.

The enzyme affinity for the oxidative pathway determined for women (1.03 mg/l) is more similar to that determined for mice (1.69 mg/l) than that determined for rats (0.1 mg/l, Gargas et al., 1990). The scaled in vivo Vmax for the oxidative pathway for women was larger than for mice and rats (25.9 vs. 10.9 and 4 mg/h-kg0.7, respectively). For GSH conjugation in the liver, the estimated VMAXgC value for humans (1.03 mg/h-kg0.7), was intermediate between the optimized values for rats and mice (0.59 and 2.61 mg/h-kg0.7, respectively). Particularly noteworthy is that the human liver VMAXgC estimates derived from scaling from two different species were within 7% of each other. This excellent concordance lends confidence to the extrapolation technique. The concordance between kidney metabolism estimates from mouse and rat was also excellent. Wheeler et al. (2001) have shown that the catalytic efficiency of rat GST 5-5 for conjugation of chloroethane is 5.7-fold higher than that of human GST-T1, which is in contrast to the similarity between the rat and human cytosolic conjugation rates of chloromethane reported by Thier et al. (1998). However, no data were available on the relative abundance of the GST 5-5 and T1 isozymes in rat versus human liver. Nor were data available on the activity of any other mammalian GSTs toward chloroethane.

The enzymatic rate of GSH conjugation in extrahepatic tissues other than kidney was estimated by adjusting for tissue weight differences between species (see Supplementary Data). Very little information is available on GST theta expression in the uteri of mice, rats, or humans. Mouse uterus GSTT2-2...
mRNA expression was found to be repressed by estrogen (Watanabe et al., 2002). GSTT1-1 and GSTT2-2 have been identified in decidua of full-term pregnant women delivering by elective Cesarean (n = 21 normal pregnancies) (Zusterzeel et al., 1999) and GSTT-1 was found in decidua from a woman with cervical cancer undergoing a hysterectomy at 13 weeks gestation (Rajmakers et al., 2001). The meaning of these results for nonpregnant women is unclear, and with no information available on GST theta in uteri of rats, no useful interspecies comparisons can be made.

The amounts metabolized via GST (Fig. 7) or cytochrome P-450 (Fig. 6) and the total amount of chloroethane absorbed (Supplementary Data, Fig. C-5) are most consistent with the observations made concerning uterine tumors, with amounts metabolized via GST providing the larger relative quantitative difference between the two rodent species. The mouse absorbs more chloroethane gas than the rat and also metabolizes more chloroethane via GST and cytochrome P-450. Humans metabolize less chloroethane via cytochrome P-450 than rats or mice on a mg/kg basis, at least up to several thousand ppm of inhaled chloroethane (Fig. 6). The continued increase in amount metabolized via cytochrome P-450 above several thousand ppm is likely due to postexposure metabolism driven by more extensive tissue storage of chloroethane in humans during exposure. The majority of the net amount absorbed (amount inhaled minus amount exhaled) under bioassay conditions is expected to be metabolized in mice and rats, as well as in humans, so a metabolite, or possibly a combination of metabolites, might be the most pertinent dose metric associated with uterine tumors in mice. It is noteworthy, however, that a direct extrapolation from the cancer effect level in mice to a possible human-equivalent effect level on the basis of GST-metabolite production cannot be made because women appear to be unable to generate sufficient flux through this pathway. If appropriate uncertainty factors were applied to the dose metric prior to interspecies extrapolation, more realistic interspecies risk comparisons could be generated.

Insights into potential mode(s) of action for the cancer effects seen in mice exposed to chloroethane can be explored using comparisons to the chemical analog, chloromethane. Chloromethane produces kidney tumors in male mice only at the highest concentration tested (1000 ppm, see Bolt and Gansewendt, 1993), and similarly, chloroethane produced uterine tumors in mice, at the highest, and only, concentration tested (15,000 ppm). Chloromethane shares similar metabolic pathways and metabolic products (Kornbrust and Bus, 1983). Both chemicals are metabolized by cytochrome P-450 leading to their respective aldehydes (acetaldehyde for chloroethane, Fedtke et al., 1994a; b; formaldehyde for chloromethane formed from GSH-derived conjugates and not primarily from P-450 metabolism, see Bolt and Gansewendt, 1993; Kornbrust and Bus, 1982, 1983). GSH is also extensively consumed in the high-dose associated metabolism of both compounds, leading to significant tissue depletions of GSH (for chloromethane see Bolt and Gansewendt, 1993; Delbanco et al., 2001; Dodd et al., 1982; Jager et al., 1988). GSH depletion, likely coupled to associated formation of toxic GSH-conjugate derived metabolites, for example, methanethiol and/or formaldehyde, has been hypothesized in mediating the toxicity of chloromethane responses (Chellman et al., 1986a; b; Kornbrust and Bus, 1983). A similar argument can be developed for the carcinogenicity of chloroethane where a perturbation in hormonal status resulting from a GSH metabolite of chloroethane may work in concert with acetaldehyde at very high inhaled concentrations, leading to the uterine tumors in mice. This hypothesis is described more thoroughly below.

Potenger et al. (1992) hypothesized that chloroethane-induced hyperactivity reported in mice from her work and that of the NTP (1989) may be related to the formation of uterine tumors. Potenger furthermore demonstrated that the species-, sex-, and dose-dependent hyperactivity was associated with chloroethane metabolism via the GSH pathway, as the hyperactivity was absent in mice that had been treated with buthionine sulfoxime, a GSH depleting agent, prior to chloroethane exposures normally sufficient to elicit the murine hyperactivity. Interestingly, chloromethane, in addition to the similarities described above, has been shown to lose its neurototoxic effects when GSH is depleted prior to exposures normally resulting in neurotoxicity or death (Chellman et al., 1986a). Endometrial uterine tumors of the type identified in the NTP study are well known to be caused by hormonal imbalances in experimental animals (Deerberg and Kaspareit, 1987; Morikawa et al., 1982; Tang et al., 1984) as well as in humans (Judd et al., 1980; Mack et al., 1976; Meissner et al., 1957). Another proposal is that estrogen unopposed by sufficient progesterone, such as occurs in constant estrus, can lead to inappropriate stimulation of the endometrium and eventual tumorigenesis (Key, 1995; Möllerstrom et al., 1993; Potischman et al., 1996; Zeil, 1982). Bucher et al. (1995) reported on serum hormone levels in young female mice exposed to either chloroethane or bromoethane via inhalation for six weeks, and did not find any statistically significant differences compared with controls. However, the distribution of treated mice across the estrous cycle stages was not equal across treatment groups, such that the number of mice for each stage varied from two to 11, complicating comparisons. Also, large variations in measured values, with standard errors of about 20–70% of the mean values, made statistical comparisons difficult, thus these data cannot be said to refute the hypothesis described above.

Also well known is that the central nervous system (CNS) regulates hormonal processes that then can have impact on the adrenals and the reproductive system, and thus chemically induced CNS dysfunction offers a potential route to reproductive organ dysfunction. In this scenario chronic inhalation exposure to extremely high concentrations of chloroethane (15,000 ppm), which causes CNS dysfunction manifested acutely and chronically as hyperactivity selectively
in female mice, may lead to alterations of the hypothalamic-pituitary axis (HPA), resulting in secondary physiological and immune dysfunctions with the potential to promote uterine tumorigenesis. There are a number of examples of stress, HPA axis involvement, and adrenocorticotropin hormone mediated cancer related events (Haller et al., 1999; Pare et al., 1999; Rivier, 1999; Reiche et al., 2004; Spiegel et al., 2006; Vrezas et al., 2003). Thus, the hypothesis proposed by Potter et al. (1992), argues that the uterine tumors observed in chloroethane-exposed female B6C3F1 mice were secondary to the effects of a GSH-derived chloroethane metabolite on the CNS, rather than a direct effect of chloroethane on endometrial DNA. The lack of direct DNA binding demonstrated by in vitro studies supports a secondary mechanism (Kornbrust et al., 1985). The PBPK modeling results reported here are consistent with the hypothesis that a GSH-derived metabolite of chloroethane, possibly in association with acetaldehyde, is involved with the mode of action for uterine tumors in mice.

SUPPLEMENTARY DATA

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

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