A Toxicokinetic Study of Inhaled Ethylene Glycol Monomethyl Ether (2-ME) and Validation of a Physiologically Based Pharmacokinetic Model for the Pregnant Rat and Human

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Exposures to sufficiently high doses of ethylene glycol monomethyl ether (2-methoxyethanol, 2-ME) have been found to produce developmental effects in rodents and nonhuman primates. The acetate metabolite of 2-ME, 2-methoxyacetic acid (2-MAA), is the likely toxicant, and, as such, an understanding of the kinetics of 2-MAA is important when assessing the potential risks to humans associated with 2-ME. A previously described physiologically based pharmacokinetic (PBPK) model of 2-ME/2-MAA kinetics for rats exposed via oral or iv administration was extended and validated to inhalation exposures. Pregnant Sprague-Dawley rats were exposed for 5 days (gestation days 11–15), 6 h/day, to 2-ME vapor at 10 and 50 ppm. Validation consisted of comparing model output to maternal blood and fetal 2-ME and 2-MAA concentrations during and following 5 days of exposure (gestation days 11–15). These concentrations correspond to a known no observed effect level (NOEL) and a lowest observed effect level (LOEL) for developmental effects in rats. The rat PBPK model for 2-ME/2-MAA was scaled to humans and the model (without the pregnancy component) was used to predict data collected by other investigators on the kinetics of 2-MAA excretion in urine following exposures to 2-ME in human volunteers. The partially validated human model (with the pregnancy component) was used to predict equivalent human exposure concentrations based on 2-MAA dose measures (maximum blood concentration, Cmax, and average daily area under the 2-MAA blood concentration curve, AUC, during pregnancy) that correspond to the concentrations measured at the rat NOEL and LOEL exposure concentrations. Using traditional PBPK scale-up techniques, it was calculated that pregnant women exposed for 8 h/day, 5 days/week, for the duration of pregnancy would need to be exposed to 12 or 60 ppm 2-ME to produce maternal 2-MAA blood concentrations (Cmax or average daily AUC) equivalent to those in rats exposed to the NOEL (10 ppm) or LOEL (50 ppm), respectively. © 2000 Academic Press

Key Words: 2-Methoxyethanol; 2-methoxyacetic acid; physiologically based pharmacokinetic (PBPK) model.

Short chain ethylene glycol monoethers have found multiple uses as solvents because of their ability to form solutions with both water and less polar organic materials. The ethylene glycol monoethers formed with methyl and ethyl groups (ethylene glycol monomethyl ether, EGME [2-ME] and ethylene glycol ethyl ether, EGEE [2-EE]) and the acetate ester of EGEE (EGEEA) were used extensively in the past for various solvent applications, including coatings applications, cleaning solvents, and EGME, in particular, as a military jet fuel additive for deicing purposes (American Conference of Governmental Industrial Hygienists [ACGIH], 1991). In the past 10 to 15 years domestic (US) markets for these glycol ethers have greatly diminished. For jet fuel additive use, 2-ME has been largely replaced with its diethylene glycol analog. In general, producers of 2-ME, EGEE, and EGEEA warn against their use in consumer products. In addition, in the US there has been an effort to replace 2-ME, EGEE, and EGEEA as components in photoresist formulations used in microelectronics industry. The primary use of 2-ME is as a process/extraction solvent in pharmaceutical production units and as a chemical intermediate in the production of glymes (dimethyl ethers of ethylene glycols; mono-, di-, and tri-).

2-ME is a developmental toxicant in all tested laboratory animals, including nonhuman primates (Nagano et al., 1981; Hanley et al., 1984; Scott et al., 1989; Paustenbach, 1988). 2-ME is metabolized in rodents to ethylene glycol (EG), via microsomal oxidation, and to 2-methoxyacetic acid (2-MAA), via alcohol and aldehyde dehydrogenase (Foster et al., 1984) (see Fig. 1). Based on both in vivo and in vitro evidence,
2-MAA has been identified as the proximate developmental toxicant in all species tested (Miller et al., 1983; Brown et al., 1984; Yonemoto et al., 1984; Ritter et al., 1985).

The current permissible exposure limit (PEL) for occupational exposure, established by the OSHA in 1977, is 25 ppm 2-ME, 8-h time-weighted average (TWA) (NIOSH, 1997) with a skin notation. This standard was established on the basis of blood, kidney, liver, and central nervous system toxicity. OSHA has proposed a PEL of 0.1 ppm (TWA) for 2-ME based on reproductive and developmental toxicity (OSHA, 1993). The threshold limit value (TLV) established by the ACGIH is 5 ppm (TWA) for 2-ME (ACGIH, 1999) and was based on findings of testicular toxicity in shipyard workers (ACGIH, 1991).

Close evaluation of the dosimetry–toxicity relationship has provided evidence that, during pregnancy, the total exposure to 2-MAA (AUC levels), or peak 2-MAA concentrations (C_{max}), appear to be well-correlated to developmental toxicity following exposure to 2-ME; the better metric depended on the endpoint being evaluated (Clarke et al., 1992; Terry et al., 1994). Developmental effects are known to be very sensitive to timing with respect to the type of effect and severity. However, for most systemic toxicants, at nearly all doses that will be encountered in the workplace or ambient environment, it is the average circulating blood level (internal dose) that will be the best metric, as is generally true for many pharmaceutical agents (Gilman et al., 1990). If there is a possibility that exposures for a sufficient period of time, for example, several hours or days, raise the blood concentration to extraordinarily high levels, then peak blood concentration may be a better dose metric, but this should rarely be encountered in most settings. As with virtually all chemicals, quantitation of internal dose, under a variety of exposure conditions, is required to most properly assess the risk to humans who might be exposed to 2-ME.

Several physiologically based pharmacokinetic (PBPK) models have been developed to describe the kinetics of 2-ME and 2-MAA in mice and rats following oral and iv exposures (Clarke et al., 1993; Terry et al., 1995; Welsch et al., 1995; Hays et al., 2000). Welsch et al. (1995) also developed an initial description of an inhalation model for 2-ME and 2-MAA kinetics in pregnant women and compared the peak plasma concentrations predicted by the model for airborne concentrations of 0.1 ppm (the proposed PEL; OSHA, 1993) with plasma concentrations in mice known to result in developmental toxicity (plasma concentration >1 mM). They determined that occupational exposures at 0.1 ppm resulted in a plasma concentration in humans of about 1 μM, or 1000-fold below that which is known to cause developmental toxicity in laboratory animals. The human model described by Welsch et al. (1995) was not verified with data nor was the fetal subcompartment developed for the mouse amenable for extrapolation to humans, limiting confidence in the model for risk assessment. A more recent PBPK model for 2-ME in the rat (Hays et al., 2000) simplified the description of the fetal compartment and is better suited for extrapolation to humans. This model has also been validated for oral and iv exposures in the rat.

There are four objectives to the work reported here: 1) to conduct a pharmacokinetic study in the rat that provides data sufficient to reinforce and validate the model of Hays et al. (2000); 2) to extend the oral/iv pregnant rat model for 2-ME to include the inhalation route; 3) to further develop the inhalation model for 2-ME in pregnant women; and 4) to determine the concentration of 2-ME in air that results in human internal doses of 2-MAA that are equivalent to the internal doses (blood concentrations) associated with known no observed effect level (NOEL) and lowest observed effect level (LOEL) for developmental toxicity that was determined for rats.

**METHODS**

**PBPK Model—Rat**

The model of Hays et al. (2000) was used to make the predictions reported in this paper. Briefly, the model for 2-ME was based on an approach reported by Fisher et al. (1989) in a PBPK model that was developed to describe trichloroethylene disposition in the pregnant rat. The basic model includes compartments representing the liver, fat, and slowly and richly perfused tissues (Fig. 1). However, three additional tissue masses need to be accounted for during pregnancy: 1) mammary tissue, 2) placental tissue, and 3) fetal tissue. The approach adopted in this model was the inclusion of the developing fetuses and the placental tissue into the richly perfused compartment and the mammary tissue into the fat compartment. This simplification was based on the observation that concentrations of 2-MAA in rat fetuses are nearly identical (or proportional) to measured maternal rat blood concentrations of 2-MAA, as well as to predicted concentrations of 2-MAA for the richly perfused tissues following both oral and iv doses of 2-ME or 2-MAA (Clarke et al., 1993; Terry et al., 1995; Welsch et al., 1995; Hays et al., 2000). Previous efforts to model the pharmacokinetics of 2-ME in the rat following iv and oral doses indicated that the combining of the fetal and placental tissues with the richly perfused compartment of the dam was justified. While it is preferable to include a discrete description of the developing embryo/fetus and the pregnancy-associated changes in the mother, the difficulty lies in developing parameters for these complex models and how to validate them in humans. Therefore, the approach of grouping these tissues allows for extrapolation to humans that requires fewer assumptions and uncertainties, qualities desirable for risk assessment.

The measured tissue/blood partition coefficients for 2-MAA are approximately 1.0 for all tissues (including fetal tissue), since 2-MAA is highly water soluble. The only exception is adipose tissue, where the tissue/blood partition coefficient in rats is 0.05 (Hays et al., 2000). Based on the above, it is reasonable to mathematically combine the fetal and placental tissues with the richly perfused compartment and the mammary tissue with the fat compartment of the dam. The procedures used by Fisher et al. (1989) to describe the changes in mammary, placental, and fetal tissue during pregnancy were also used here. Hays et al. (2000) determined the slowly perfused tissue/blood partition coefficient (SPC) for 2-MAA by optimization. Using a SPC of 1.0, the model did not accurately predict plasma levels of 2-MAA after iv administration of the compound, and using a SPC value of 0.5 substantially improved the fit. The authors attribute this discrepancy between in vitro and in vivo findings to difficulties homogenizing slowly perfused tissues.

The fractional respiratory bioavailability (\( K_{aw} \)) was applied to the alveolar ventilation rate. It was not a correction for dead space or a conversion between
2-ME
Inhaled air
Exhaled air

Lung
Fat
Richly Perfused
Poorly Perfused
Liver

2-MAA

Venous Blood
Arterial Blood

Urine

EG Kegc

Kmaac

FIG. 1. The structure of the PBPK model describing 2-ME and 2-MAA kinetics in rats and humans (modified from Hays et al., 2000).

Concentration (Total daily exposure durations were 6 h plus the time to reach 90% of the target 50 ppm 2-ME in stainless steel and glass Hazelton 2000 inhalation chambers. Consecutive days (gestation days 11–15) to targeted concentrations of 10 and 20 ppm was observed twice daily for moribundity and mortality and body weights were determined prior to the first and last day of exposure.

Animals. Timed pregnant Sprague–Dawley Crl:CD rats were obtained from Charles River (Raleigh, NC). Animals were fed Purina 5002 certified pelleted diet and municipal tap water ad libitum except during exposure, when only water was provided. All animals were housed individually in the Hazelton 2000 chambers (Hazelton, Aberdeen, MD) used for exposures. Each animal was observed twice daily for moribundity and mortality and body weights were determined prior to the first and last day of exposure.

Exposure conditions and chamber monitoring. Animals were exposed 5 consecutive days (gestation days 11–15) to targeted concentrations of 10 and 50 ppm 2-ME in stainless steel and glass Hazelton 2000 inhalation chambers. Total daily exposure durations were 6 h plus the time to reach 90% of the target concentration ($T_{90}$). $T_{90}$ averaged 18 min for 2-ME. The airflow, temperature, and relative humidity of each chamber was maintained at approximately 425 liters/min, 75°F, and 55%, respectively.

Atmospheres of each test material were produced using a Battelle-designed vapor generating system. The concentration of 2-ME in each chamber was controlled by adjusting the test material pump rate and dilution airflow. The distribution of test material over 12 sampling sites within each chamber was verified to be ±5% relative standard deviation.

Chamber concentrations were determined approximately 15 times/exposure period by gas chromatography/flame ionization detection (GC/FID) (Hewlett Packard 5890; Palo Alto, CA) using a 30 m × 0.53 mm id, RTX-5, 5-μm film capillary column (Restek Corp., Bellefonte, PA). Helium was used as the carrier gas at a head pressure of ~6 psi. The column was maintained isothermally at 150°C. Under these conditions the retention time for 2-ME was ~0.9 min.

A VALCO stream select valve constructed of Hastelloy-C (VALCO Instrument Co., Inc., Houston, TX) provided the interface between the on-line gas chromatograph, the exposure chamber, and an on-line diffusion tube standard (Model 491; Kin-Tek Laboratories, Inc., La Marque, TX), which provided a constant vapor concentration of 2-ME. This stream select valve directed a continuous stream of sampled atmosphere to a six-port, VALCO sampling valve (Hastelloy-C) with a 0.1-ml sample loop. Both valves were mounted in a dedicated valve oven and heated to 150°C.

The operation of the exposure monitor was checked throughout the day against the on-line standard to check for drift in the on-line monitor calibration. Additional calibration checks (by grab sampling) were performed when significant drift of the on-line GC response factor was indicated by a shift in the on-line standard concentration. Precision of the on-line monitor was assessed by taking five successive readings at each sample port. For 2-ME, the largest relative standard deviation observed for successive readings at a single port was 1.8%.

Analytical methods for rat blood, urine, and fetuses. An analytical method was developed to simultaneously quantitate 2-ME and 2-MAA in rat blood, urine, and fetuses. The method involved acidification of 0.5 g of blood, urine, or pooled (by litter) fetal homogenates with an equal volume of 0.9 M sulfuric acid, addition of 0.25 g sodium sulfate (to improve extraction efficiency), and extraction with 0.5 ml ethyl acetate. Separation and quantitation was achieved using a 30 m × 0.32 mm id × 0.5-μm film thickness Stabilwax DA capillary column (Restec) and a Hewlett Packard 6890 GC with FID.
Spitless injections of $1.5 \mu L$ of extract were made at an injector temperature of $200^\circ C$. The initial oven temperature was $70^\circ C$ for 2.5 min and then was increased at $20^\circ C/min$ to $240^\circ C$ and held for 1 min. Hydrogen was used as the carrier gas at 18 psi constant pressure. The FID temperature was $270^\circ C$.

To achieve the sensitivity and selectivity needed to simultaneously analyze 2-ME and 2-MAA, two modifications to the standard capillary GC/FID system were required. First, a Cyclo double gooseneck injection liner (Restek) was used to concentrate the sample and to provide an initial sample clean-up step prior to chromatography. Second, a 25-cm section of deactivated 0.25 mm id capillary column was connected to the Stabilwax-DA column prior to the detector to improve peak shape for quantitation. Ethoxyethanol and ethoxyacetic acid were used as internal standards for 2-ME and 2-MAA analyses, respectively. All standard curves were generated using matrix-spiked standards to correct for extraction efficiencies. Detection limits for 2-ME and 2-MAA were approximately 0.012 $\mu g/L$ of extract were made at an injector temperature of $200^\circ C$. The initial oven temperature was $70^\circ C$ for 2.5 min and then was increased at $20^\circ C/min$ to $240^\circ C$ and held for 1 min. Hydrogen was used as the carrier gas at 18 psi constant pressure. The FID temperature was $270^\circ C$.

Blood sampling and analyses. Blood samples were collected via cardiac puncture from each rat under anesthesia (sodium pentobarbital) on the last day of five consecutive exposures (gestation day 15), immediately frozen, and stored in borosilicate glass containers at $-70^\circ C$ until analyzed. Blood samples were collected from four rats/sampling time at 1 and 3 h during the exposure, immediately post-exposure, and at 0.5, 1, 2, 4, 8, 18, 42, 66, and 90 h postexposure. Blood samples were collected from control animals, spiked with known quantities of 2-ME and 2-MAA, and stored frozen along with the samples collected from the exposed animals to correct for potential losses of analytes during storage. Blood samples were analyzed, in duplicate, for 2-ME and 2-MAA by GC/FID.

Urine sampling and analyses. Each rat scheduled for the 18-h postexposure blood collection time point was placed in individual metabolism cages (Lab Products, Seaford, DE) for the 0–18-h postexposure urine collection. Each animal was provided access to water but not feed. Urine samples were collected over dry ice and stored at $-70^\circ C$ until analyzed, in duplicate, as described above. Control urine samples were spiked with appropriate analytes and stored frozen to correct for potential losses during storage.

Fetal sampling and analyses. The uterus was removed from each rat at the time of blood collection. Each fetus was removed, placed as a litter in a single container, weighed, and flash frozen in liquid nitrogen. With blood and urine samples, control fetal samples were homogenized, spiked with appropriate analytes, and stored frozen to correct for potential losses during storage. Only fetuses collected at 0 and 8 h postexposure were analyzed for 2-ME and 2-MAA as described above.

PBPK Model for Pregnant Women

The PBPK model that was used to make predictions for exposure of 2-ME to pregnant women follows the same general structure (with respect to number of compartments, growth of compartments, and sites of metabolic activity) as for the pregnant rat (Fig. 1). As with the rat model, the mammary tissue was grouped with the adipose tissue and the placenta and fetuses were combined in the richly perfused compartment for the human model. However, the physiological parameters are those typical of a 58-kg human female, and the empirical equations describing growth of the various compartments over the 40 weeks of pregnancy have been taken from other sources (Table 1). The values listed in Table 1 are representative of the beginning of pregnancy.

The partition coefficients determined in rats were used as surrogates for human tissues, with the exception of the use of human blood:air partition coefficients (Johanson and Dynesius, 1988). This approach is supported by the findings that tissue:blood partition coefficients for 2-ME and 2-MAA in rat and mouse tissues are very similar—2-ME partition coefficients in rat were $96 \pm 16\%$ of the partition coefficients in mouse tissues. The blood:air partition coefficients for 2-ME are very similar among the three species (rat, 31,300; mouse, 34,900; human, 32,800; Hays et al., 2000; Clarke et al., 1993; Johanson and Dynesius, 1988).

The kinetic constants of metabolism of 2-ME to 2-MAA and to EG were determined from the data of Green et al. (1996) (see Table 1). The rate constant describing the urinary excretion of 2-MAA ($K_{\text{urin}}$) was determined by fitting model output to the data from Groeseneken et al. (1989). As described by Hays et al. (2000), $K_{\text{ex}}$ and the slowly perfused tissue:blood partition coefficients were the only model parameters that were adjusted for the rat. The only model parameter that had to be “fit” in the human model was $K_{\text{urin}}$. All other parameters were measured or taken from the literature.

Estimating Equivalent Internal Doses

The PBPK model for the rat was used to predict the expected peak ($C_{\text{mt}}$) and average daily area under the blood concentration curve (AUC) for 2-ME in the rat under the conditions of the critical toxicology study (Hanley et al., 1984). That is, the rats were exposed 6 h/day on GD 6–15. $C_{\text{ex}}$ and the average daily blood AUC of 2-MAA during GD 13–15 were computed. The choice of GD 13–15 was based on the experimental conditions that maximize the occurrence of malformations and number of live embryos/litter (Sleet et al., 1996) in rats dosed with 500 mg 2-ME/kg body wt iv. These same values for $C_{\text{ex}}$ and average daily AUC in the rat were used as the target values for predictions using the human pregnancy model. Various inhaled concentrations of 2-ME for 8-h periods, 5 days/week for the 40 weeks of pregnancy, were used as inputs to the human model until $C_{\text{ex}}$, and average daily AUC values for 2-MAA in blood were equal to those determined for rats. The resulting inhaled 2-ME concentrations for humans were then considered to be equivalent to the NOEL and LOEL in rats for developmental effects, based on these internal doses.

All modeling was performed using the Advanced Continuous Simulation Language (ACSL; MGA Associates, Inc., Cambridge, Massachusetts).

RESULTS

Animal Experiments

The average concentrations of 2-ME over the 30 h of exposure (over 5 days) in the chambers were 10.7 and 47.2 ppm for the target concentrations of 10 and 50 ppm, as determined by GC/FID. The 50-ppm chamber exposures were more variable than expected with daily concentrations ranging from 34.5 ppm (third exposure) to 59.2 ppm (fifth exposure) as a result of malfunctions in the vapor generating system. The temperature and relative humidity of the chambers averaged 100–101 and 95–100% of target, respectively. There were no differences observed between exposure groups in body weights. In addition, no treatment-related clinical signs or changes in general appearance or behavior were observed in the rats at any exposure.

Prediction of Rat Data

Since the half-life of 2-MAA in urine is on the order of 24 h in rats (Medinsky et al., 1990), exposures to 2-ME were conducted over a 5-day period to allow near steady-state conditions to be achieved. Preliminary modeling indicated that five exposures of 6 h/day was sufficient. An array for the measured exposure concentrations was used as input to the model to allow for different exposure concentrations on each of the exposure days, since the actual concentrations of 2-ME in the inhalation chamber differed slightly from the targeted concentrations.
The body weights of the rats were increasing during the exposure period due to pregnancy. Therefore, the body weights measured at necropsy at each time point (after the 5 days of exposure) were used to derive a linear regression line describing the body weight of each exposure group (i.e., 10- and 50-ppm-exposed animals). The resulting regression lines for

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Parameter estimation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weights</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight-10-ppm-exposed group</td>
<td>bw = 0.4705*T + 90.3</td>
<td>Measured</td>
</tr>
<tr>
<td>Body weight-50-ppm-exposed group</td>
<td>bw = 0.5296*T + 73.8</td>
<td>Measured</td>
</tr>
<tr>
<td>Body weight (start and end of pregnancy)</td>
<td>58, 62 kg</td>
<td>Fixed</td>
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<tr>
<td>Percentage of body weight:</td>
<td>GD 13</td>
<td>Day 0</td>
</tr>
<tr>
<td>Liver</td>
<td>4.0</td>
<td>2.4</td>
</tr>
<tr>
<td>Fat</td>
<td>10.1</td>
<td>27.6</td>
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<tr>
<td>Slowly perfused</td>
<td>65.0</td>
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<tr>
<td>Richly perfused</td>
<td>6.1</td>
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<tr>
<td>Blood</td>
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<td>5.9</td>
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<tr>
<td>Flows</td>
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<tr>
<td>Cardiac output (L/h/kg&lt;sup&gt;0.75&lt;/sup&gt;)</td>
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<td>19.2</td>
</tr>
<tr>
<td>Alveolar ventilation (L/h/kg&lt;sup&gt;0.75&lt;/sup&gt;)</td>
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<td>15.3</td>
</tr>
<tr>
<td>Percentage of cardiac output (%):</td>
<td>GD 13</td>
<td>Day 0</td>
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<tr>
<td>Liver</td>
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<td>24.0</td>
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<tr>
<td>Fat</td>
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<tr>
<td>Slowly perfused</td>
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<td>Richly perfused:blood</td>
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<tr>
<td>K&lt;sub&gt;maac&lt;/sub&gt; (L/h/kg liver) 2-ME → MAA</td>
<td>31</td>
<td>4.9</td>
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<tr>
<td>K&lt;sub&gt;egc&lt;/sub&gt; (L/h/kg liver) 2-ME → EG</td>
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<tr>
<td>K&lt;sub&gt;alv&lt;/sub&gt; (unitless)</td>
<td>0.76</td>
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</table>

<sup>a</sup> Body weight (g) as a function of time (hours). T = 0 at the beginning of gestation. Equation used for T > 312 h.

<sup>b</sup> Increase is divided among liver, richly and slowly perfused tissues, and blood based on initial volume fraction. Does not include fetus, placenta, and mammary tissue (ICRP, 1975).

<sup>c</sup> Includes growing mammary tissue (Knight et al., 1984; Luecke et al., 1994).

<sup>d</sup> Includes growing placenta and fetus(es) (Fisher et al., 1989; Hays et al., 2000; Luecke et al., 1994).

<sup>e</sup> From Andersen et al. (1991).

<sup>f</sup> Includes increasing flow to mammary tissue (Fisher et al., 1989; Metcalfe et al., 1988).

<sup>g</sup> Includes flow to placenta and fetus, calculated as 100% flows to other tissues.

<sup>h</sup> Head space vial equilibration with human blood, reference is air. (Johanson and Dynesius, 1988).

<sup>i</sup> Nonvolatile vial equilibration, reference is saline (Hays et al., 2000).

<sup>j</sup> Average of liver, placenta, and fetus.

<sup>k</sup> Green et al. (1996).

<sup>l</sup> Tyson et al. (1989).

<sup>m</sup> Optimized by fitting model output to blood concentrations or urinary output of 2-MAA (see text for discussion).

<sup>n</sup> Groeseneken et al. (1989).
Table 1. The regression line was used to derive an array of body weights as a function of time for modeling 2-ME and 2-MAA concentration time-courses in blood. The TABLE function of ACSL was used to interpolate and extrapolate body weights at all times of the simulation (MGA Software, 1995).

Peak blood concentrations of 0.2 and 1.3 mg/L (Fig. 2a) 2-ME were measured at the end of the final exposures on GD 15 for the 10- and 50-ppm exposures, respectively. The model overpredicted 2-ME maternal blood concentrations by about a factor of 2 at 10 ppm 2-ME and gave a reasonable prediction at 50 ppm 2-ME. For the major metabolite, 2-MAA, peak blood concentrations of 7.1 and 62.7 mg/L (Fig. 2b) were measured at the end of the 10- and 50-ppm exposures, respectively. The PBPK model provided good predictions of the 2-MAA blood concentration time-courses for both exposure concentrations.

Concentrations of 2-ME in two of the four rat fetal samples collected at the end of the last exposure were similar to their corresponding maternal blood sample concentrations (data not shown). Concentrations of 2-ME were below the limits of reliable quantitation in the remaining two fetal samples. No 2-ME was detected in rat fetuses at 8 h postexposure. 2-MAA was readily detected in all rat fetal samples with concentrations averaging 25% higher than the corresponding maternal blood concentrations at 0 and 8 h postexposure (Fig. 2b). This ratio between maternal and fetal 2-MAA concentrations was consistent following 10- and 50-ppm exposures at both the conclusion of exposure and at 8 h postexposure. This marginal increase might be attributable in part to the richly perfused: blood partition coefficient of 1.1. Since the concentration profiles of 2-MAA in rat embryos and maternal blood are proportional for both dose levels following several days of exposure, the maternal blood concentration of 2-MAA appears to be a reasonable indicator of, and surrogate for, embryo exposure to 2-MAA.

The excretion rate of 2-MAA in urine, $K_{ex}$, was determined by visual inspection, varying this parameter until an acceptable fit to the measured maternal rat blood and fetal 2-MAA concentrations was produced. This value (Table 1) was confirmed as optimal using a well-accepted optimization criterion, maximization of the log likelihood function (Steiner et al., 1990).

The model predicts that 0.55 and 2.78 mg 2-MAA will be excreted in the urine after exposure to 10 and 50 ppm 2-ME, respectively. Postexposure urinary excretion of 2-MAA was measured to be 0.16 mg for the 10-ppm exposures and 0.95 mg for the 50-ppm exposures (no free EGME was detected in any urine sample). However, the model predictions would be expected to include all 2-MAA-derived urinary metabolites, and the analytical method measures only 2-MAA. Experimental work (Sumner et al., 1995; Sabourin et al., 1992) indicates that 2-MAA-derived metabolites such as glycine conjugates may contribute 14 to 80% more 2-MAA-derived urinary metabolites. The average in studies with male F344 rats was 28%. It is therefore estimated that measured amounts of urinary 2-MAA should be multiplied by a factor of 1.3 to approximate the total flux through the 2-MAA pathway (amount of unchanged 2-MAA plus glycine conjugates and other metabolites). The resulting estimates of 0.21 and 1.24 mg 2-MAA-derived urinary metabolites are just less than half the model predictions.

Model Predictions of Human Data

Groeseneken et al. (1989) determined the pulmonary retention of 2-ME and the rate of 2-MAA excretion in urine from male human volunteers exposed to 16 mg/m$^3$ (5 ppm) of 2-ME. The exposure regimen consisted of four 50-min exposures with a 10-min break at the end of each 50-min exposure for urine collection, with urine collection continuing for 5 days. The human PBPK model described above (less the parameters used to describe pregnancy) was used to make predictions of the
urinary excretion rate data collected by Groeseneken et al. (1989) for male volunteers. The first-order urinary excretion rate constant ($K_{ex}$) was allowed to vary until a reasonable fit, as determined by visual inspection, was achieved (Fig. 3). Optimization based on maximization of the log likelihood function (Steiner et al., 1990) was not done because this procedure produced estimates of $K_{ex}$ that improved predictions of early time points but dramatically underpredicted rates at later times. All other model parameters remained fixed for this exercise. The PBPK model, with $K_{ex}$ fixed, predicted 15.6 mg of 2-MAA should be excreted during the 120 h of the exposure and postexposure periods described by Groeseneken et al. (1989). The total 2-MAA excreted as reported by Groeseneken et al. (1989) was 16.6 mg. These predictions indicated that the value of $K_{ex}$ was reasonable, even though the model slightly underpredicted the early excretion rates.

**Equivalent Internal Dosimetry**

The PBPK model for the pregnant rat was used to determine the blood $C_{max}$ and average daily AUC for 2-MAA in the animal study described here. The model was considered reliable for estimating these values based on the fits achieved for 2-MAA blood and fetus concentrations (Fig. 2b). The model was then used to predict $C_{max}$ and average daily AUC under the experimental conditions of the rat inhalation developmental toxicity study described by Hanley et al. (1984). That study defined the NOEL and LOEL for 2-ME of 10 and 50 ppm, respectively. The human pregnancy model was used to predict blood 2-MAA $C_{max}$ and average daily AUC for pregnant women exposed for 8 h/day, 5 days/week for 270 days at various inhaled concentrations of 2-ME. The human equivalent inhaled concentration to the NOEL in the rat (10 ppm) was determined to be 12 ppm using either $C_{max}$ or average daily AUC for 2-MAA. The human equivalent inhaled concentration to the LOEL in the rat (50 ppm) was determined to be 60 ppm using either $C_{max}$ or average daily AUC for 2-MAA.

**DISCUSSION**

The model described in this work produces reasonable predictions of 2-ME and 2-MAA kinetics in pregnant rats following multiday exposures to 2-ME. There are, however, some differences between model predictions and observed data. The model slightly overpredicts (about a factor of 2) the 2-ME maternal blood concentrations for rats (Fig. 2a) while still producing good fits to the 2-MAA maternal blood concentrations (Fig. 2b). The overpredictions of 2-ME in maternal blood might be due to 1) an overprediction of the amount of 2-ME absorbed via inhalation, 2) an underprediction of the rate of 2-ME metabolism occurring, and/or 3) another site of 2-ME storage not presently accounted for. We will address each of these possibilities in turn.

The model predictions were found to be more consistent with the observed maternal blood concentrations of 2-ME in rat if lower values of the alveolar absorption fraction ($K_{alv}$) are used, such as a value of 0.6 (simulation not shown). However, this would yield predictions of the maternal 2-MAA concentrations in rat blood that would slightly underestimate the experimental results. It was felt prudent to use the alveolar absorption fraction measured by Groeseneken et al. (1989) for humans since it was an experimentally derived value. Also, the alveolar absorption fraction of 0.76 for 2-ME is consistent with
pulmonary absorption fraction (0.6) reported by Johanson et al. (1986) for a related glycol ether, 2-butoxyethanol (pulmonary absorption fraction = 0.8x alveolar absorption fraction due to correction for dead space). The relevance of this value for rats is still not resolved. The amount absorbed by 2-ME inhalation could also have been overestimated due to use of standard ventilation rates. If actual ventilation rates were slower than expected, the predicted 2-ME and 2-MAA concentrations in rats would, likewise, be lower.

The model described in this paper used metabolic parameters determined in vitro by Green et al. (1996) using hepatocytes from humans and Fischer 344 rats. The model fits to maternal blood concentrations of 2-ME could be improved by assuming a larger percentage of 2-ME metabolism resulted in the production of 2-MAA vs EG. However, the model used as the basis for this work (Hays et al., 2000) that included the rate constants of metabolism that were used here was accurate in predicting maternal blood concentrations of 2-MAA in rats following 2-ME iv and oral administrations. Therefore, the values determined by Green et al. (1996) were used in these model predictions without any modifications.

The other alternative is that the enzymes responsible for metabolism of 2-ME are induced during the five daily exposures. Kawamoto et al. (1990) found that 2-ME induced alcohol dehydrogenase (ADH) activities in hepatic cytosols from rats pretreated with 2-ME (100 and 300 mg/kg body wt) dosed orally for 5 and 20 days. After five daily oral exposures to 300 mg/kg 2-ME, ADH activities were increased by approximately 12% over rats exposed to vehicle control (Kawamoto et al., 1990), and, after 20 days, ADH activities were increased approximately 50% above controls. Five daily oral doses of 300 mg/kg 2-ME would result in peak liver 2-ME concentrations of approximately 200 mg/L, based on predictions made with the model described here. The five daily inhalation exposures to 50 ppm 2-ME performed in this study would be expected to result in liver concentrations peaking around 2 mg/L. Therefore, it is unlikely that the inhalation exposures performed in this study would result in significant induction of the ADH enzymes.

The third possible reason for less than optimal model prediction is that 2-ME is stored to a greater extent either in an existing tissue or in a tissue not accounted for in the model. This seems unlikely since the model used here is essentially the same as was used to successfully predict 2-ME kinetics following either oral or iv doses of 2-ME (Hays et al., 2000) and given the partitioning characteristics of 2-ME (tissue:blood partition coefficients are approximately equal to 1.0 for all tissues except fat). Therefore, it is unlikely that 2-ME is stored to a greater extent than already accounted for in a particular tissue.

Despite minor differences between the model predictions and the observed data, it should be stressed that the fits produced in these studies are quite good considering they represent exposures to 2-ME over a 5-day period. The ability of a PBPK model to be within a factor of 2 after 5 days of exposure is very good considering the parent compound is rapidly eliminated and the metabolite is significantly more long-lived. Few PBPK models that exist today have been validated with data from multiday exposure studies as was described here (Paustenbach et al., 1984).

We consider the human model described here as being partially validated since there was only a single human data set that could be used for comparison to model prediction (Groeseneken et al., 1989) and those data were used to set one of the model parameters. It is reassuring, however, that parameters used in our model, such as the rate constants of metabolism and the alveolar extraction fraction, were determined from human studies and not extrapolated from animal experiments. In addition, model predictions of the urinary excretion rates of 2-MAA, the putative active form of 2-ME, are quite good even though $K_{ex}$ was estimated from these predictions. The model did, however, accurately predict the total amount of 2-MAA excreted by human subjects during and following 2-ME exposure, thus providing partial validation of the human model. It is important to “test” a model with data not used to fit or estimate certain parameters, but in the case of human models this is not frequently possible. Still, the use of human models, even partially verified, is preferred over less scientifically robust extrapolation procedures.

The human equivalent exposure concentrations that were estimated in this work were 20% higher than exposure concentrations corresponding to the rat NOEL and LOEL. The similarity of the animal and human equivalent NOEL concentrations is somewhat unexpected. This occurrence is due to offsetting differences in human and rat toxicokinetics. While the half-life of 2-MAA is longer in humans (urinary half-life of 77 h) (Groeseneken et al., 1989) than in rats (blood elimination half-life of ~24 h, this study), humans do not convert 2-ME to 2-MAA as rapidly in the liver (clearance of 4.9 L/h/kg of liver for humans, 31 L/h/kg of liver for rats). Furthermore, due to physiological differences between rats and humans, a 250-g rat inhales 20 L air/h/kg body wt while a 70-kg human inhales 5 L/h/kg body wt. As a result, when rats and humans are exposed to the same airborne concentration (ppm) of a compound, the rat receives a higher dose on a mg/h/kg body wt basis.

Welsch et al. (1995) reported that maternal plasma concentrations of 2-MAA > 1 mM in pregnant mice results in developmental toxicity. The peak 2-MAA concentrations in rat blood at 50-ppm inhalation exposures to 2-ME (rat LOEL) were measured to be 62.7 mg/L (0.7 mM), which corresponds well to the mouse results reported by Welsch et al. (1995). The work reported here indicates that pregnant women exposed to inhaled concentrations of 2-ME for 8 h/day, 5 days/week for the duration of pregnancy do not reach blood 2-MAA concentrations that are known to be developmentally toxic to mice and rats until the exposure concentration reaches 60 ppm or higher.
The use of PBPK models has not yet become an integral part of chemical risk assessments designed to aid in developing regulatory standards regarding toxicants in air, water, food, soil, and other media (Hays et al., 1998). This has been a bit surprising since one of the primary objectives in toxicology and regulatory decision making is to be sure that all of the relevant information is brought together in a cohesive manner to bring clarity to the risk assessment. The recent EPA cancer guidelines (U.S. EPA, 1996) clearly stress the importance of selecting the proper dose metric, given the mode of toxic action for a chemical. The guidelines also place emphasis on its importance to give a high degree of confidence in the process of developing the likely dose–response relationship for humans. We are hopeful that the methods described in this article will be useful for quantitatively predicting the human risks associated with exposure to other developmental toxicants and that the results will be incorporated in regulatory decision making.

REFERENCES

American Conference of Governmental Industrial Hygienists (ACGIH). (1991). Documentation of the Threshold Limit Values and Biological Exposure Indices. 6th Ed. ACGIH, Cincinnati, OH.

American Conference of Governmental Industrial Hygienists (ACGIH) (1999). 1999 TLVs and BEIs: Threshold Limit Values for Chemical Substances and Physical Agents Biological Exposure Indices. ACGIH, Cincinnati, OH.


