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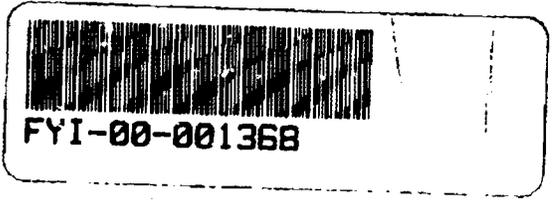
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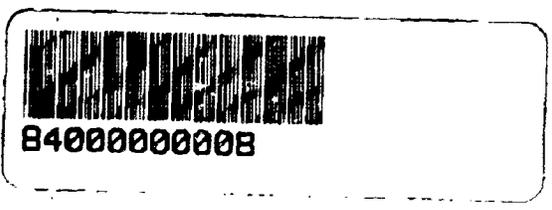
Union Carbide Corporation, Inc. "Union Carbide" herewith submits the following prepublication manuscripts concerning 2-methoxy ethanol (CASRN 109-86-4), ethylene glycol ethyl ether (CASRN 110-80-5) and ethylene glycol ethyl ether acetate (CASRN 111-15-9) which EPA may find of interest.

"Development of a Physiologically Based Pharmacokinetic Model of 2-Methoxyethanol and 2-Methoxyacetic Acid Disposition in Pregnant Rats", S. M. Hays, B. A. Elswick, G. M. Bulmenthal, F. Welsch, R. B. Conolly and M. L. Gargas.

"Validation of a Physiologically-Based Pharmacokinetic Model of Inhaled Ethylene Glycol Monomethyl Ether in the Pregnant Rat and Extrapolation to People", S. M. Hays, T. R. Tyler, R. A. Corley, K. K. Weitz, T. J. Mast, L. M. Sweeney and M. L. Gargas.

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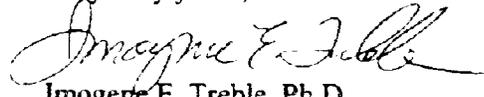
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"Development of a Physiologically-Based Pharmacokinetic Model for the
Glycol Ethyl Ether Acetate and Ethoxy Acetic Acid Kinetics in Rats"
M. L. Gargas, T. R. Tyler, R. A. Corley, K. K. Weitz, T. J. Mast, L. M. Swenberg,
and S. J. Hays.

These manuscripts are being prepared for publication in the open literature.

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**DEVELOPMENT OF A PHYSIOLOGICALLY BASED PHARMACOKINETIC
MODEL OF 2-METHOXYETHANOL AND 2-METHOXYACETIC ACID
DISPOSITION IN PREGNANT RATS**

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ABSTRACT

An accurate description of developing embryos' exposure to a xenobiotic is a desirable component of mechanism-based risk assessments for humans exposed to **potential developmental toxicants** during pregnancy. 2-Methoxyethanol (2-ME), a **solvent used in the manufacture of semi-conductors, is embryotoxic and teratogenic in all species tested including non-human primates.** 2-Methoxyacetic acid (2-MAA) is the primary metabolite of 2-ME and the proximate embryotoxic agent. The objective of the work described here was to **adapt an existing** physiologically-based pharmacokinetic model that accurately describes the pharmacokinetics of 2-ME and 2-MAA during mid-organogenesis **in mice and extrapolate that model** to rats on gestation days (GD) 13 and 15. **Those two chemicals were administered by** gavage or i.v. bolus **injections.** Blood and tissue data were simulated using a refined **and simplified** PBPK model containing actual tissue compartments for 2-ME and 2-MAA distribution. The model includes a description of the growth of the developing **embryo** and physiological and anatomical changes in the dam associated with pregnancy. Tissue partition coefficients for 2-ME and 2-MAA were determined for a variety of maternal tissues and the **embryos.** The **embryos** and associated placentas were designated as part of the richly perfused compartment of the dam because of the similarity in partition coefficients of 2-ME and 2-MAA in these tissues and the similarity in 2-MAA pharmacokinetics in maternal plasma and **conceptus** tissues following 2-ME or 2-MAA administration. Biotransformation **pathways** of 2-ME to **either** ethylene glycol (EG) **or** to 2-MAA **were** described as first-order processes based on the data collected for rats by Green *et al.* (1996). The **predictions of the model simulations closely reflected biological measurements** of 2-ME and 2-MAA **concentrations in blood and tissue.** Such data were not used for model calibration following either 2-ME or 2-MAA administration (What does this mean?). This revised and validated PBPK model of 2-ME and 2-MAA pharmacokinetics

in pregnant rats can now serve as the foundation for development of a similar model for **occupational 2-ME exposure of pregnant women.**

INTRODUCTION

Pregnant women are exposed to a **myriad of chemicals that are present in the human habitat** and in the workplace. **The toxicity potential of the materials on the developing embryo/fetus is likely to vary widely and all too often is unknown.** During pregnancy, the **physiological parameters** of the mother and **conceptus** change rapidly, and these dynamic alterations may **exert substantial impact on the pharmacokinetics of chemicals in both mother and embryo/fetus.** Evaluation of the pharmacokinetics of chemicals under conditions of exposure which elicit developmental toxicity in pregnant laboratory animals can contribute to a better understanding of the processes that govern the exposure of the target tissue (embryo) to the proximate toxic agent. A physiologically-based pharmacokinetic (PBPK) model **that integrates chemical specific parameters with changes in tissue/organ volumes and blood flow during pregnancy may be applicable to provide quantitative descriptions of the toxicant concentrations to which an embryo is exposed to during critical periods of development. Once validated in animals, such a model might be used for extrapolations to humans to predict maternal tissue and embryo dosimetry thus and providing more scientifically justified better-founded risk management alternatives for pregnant women exposed to that chemical and maybe other substances, too, that occur in the environment and workplace.**

2-Methoxyethanol (2-ME), an ethylene glycol ether which is moderately volatile, hydrophilic, and slightly lipophilic, is teratogenic in all animal species tested, including primates (Hanley *et al.*, 1984; Nagano *et al.*, 1984; Scott *et al.*, 1989). 2-Methoxyacetic acid (2-MAA) is the oxidative metabolite of 2-ME and the putative proximate toxicant

(Brown *et al.*, 1984; Yonemoto *et al.*, 1984; Ritter *et al.*, 1985; Sleet *et al.*, 1988). The teratogenic effects of 2-ME **display high developmental phase specificity**. Exposure of pregnant mice to 2-ME or 2-MAA on gestational days (GD) 7-9 produces failure of anterior neural pore closure **resulting in** exencephaly with highest susceptibility on GD 8 (Terry *et al.*, 1994). Treatment during later stages of embryogenesis (GD 9-12) affects the limb buds and produces digit malformations (Horton *et al.*, 1985).

The pharmacokinetics of 2-ME and 2-MAA **in pregnant mice and their conceptuses** have been well-defined (Clarke *et al.* 1993, Terry *et al.*, 1995). It appears as if the dosimetry-teratogenicity-relationship changes between various stages of embryogenesis. Exencephaly is best associated with peak concentrations (C_{max}) of 2-MAA in maternal plasma and embryonic compartments on GD 8 (Terry *et al.*, 1994), while digit malformations are better correlated with the area under the concentration-time curve (AUC) of 2-MAA in maternal plasma and embryonic compartments on day 11 (Clarke *et al.*, 1992). These differences in dose-response relationship and pharmacodynamics underscore the need for a thorough understanding of the pharmacokinetics of 2-ME and 2-MAA in the mother and developing embryo. Clarke *et al.* (1993) first developed a PBPK model for 2-MAA dosimetry in mice on GD 11. The model of the physiology of rodent gestation developed by O'Flaherty *et al.* (1992) served as the template onto which a description of 2-ME/2-MAA pharmacokinetics in the pregnant mouse was superimposed. The resulting PBPK model successfully simulated the pharmacokinetic behavior of 2-ME and 2-MAA in maternal plasma, embryo and extraembryonic/amniotic fluid (EAF). More recently, the model was expanded to describe pharmacokinetics on GD 8, 11, and 13, and several alternative hypotheses were proposed that help explain the **very modest accumulation of 2-MAA in GD 11 embryos and EAF compared to maternal plasma concentrations** (Terry *et al.*, 1995).

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One of the limitations of the original mouse gestation PBPK model cited above was the use of a non-physiological two compartment pharmacokinetic (PK) model to describe 2-MAA disposition. **It remains unclear how to scale such a rodent model to predict 2-ME and 2-MAA kinetics in pregnant women, although pilot simulations were performed and looked encouraging (Welsch et al., 1995) {Nothing can be said about how encouraging the simulations looked since no human data sets were simulated}.** Therefore, the work reported here describes some recent modifications to the original mouse PBPK model and provides an illustration as to how the new approach was applied to simulate the biological data collected in pregnant rats. In this communication, we show a PBPK model that describes the disposition of 2-ME and 2-MAA in rats exposed to either 2-ME or 2-MAA by i.v. injection or gavage on GD 13 and 15. The data provide the means to facilitate eventual extrapolation of the model to humans.

MATERIALS AND METHODS

Animals: Timed pregnant Crl:CD BR VAF/Plus (CD) rats (sperm positive = GD 0) were purchased from Charles River Breeding Laboratories (Raleigh, NC). They arrived one day prior to treatment, *i.e.*, on GD 12 or GD 14, and were housed overnight in a mass air displacement room (Bioclean, Hazleton Systems, Vienna, VA), maintained at 22 ± 1.5 C and $50 \pm 10\%$ humidity with a 12 hr light/12 hr dark cycle with the light cycle beginning at ~~7:00 hr~~ 7:00 a.m.. The pregnant females were singly housed and had free access to NIH 07 open formula pelleted food (Zeigler Brothers, Gardner, PA) and filtered tap water (Barnsted Nanopure System, Boston, MA).

Chemicals

Authentic 2-ME and 2-MAA (each greater than 99% pure) were purchased from Aldrich Chemical Co., Milwaukee, WI and Eastman Kodak Co., Rochester, NY, respectively. [1-¹⁴C] 2-ME (specific activity 15 mCi/mmol, radiochemical purity 99%) and [1-¹⁴C] 2-MAA (specific activity 5.0 mCi/mmol, radiochemical purity 98%) were custom synthesized (Wizard Laboratories, Davis, CA).

Tissue/Organ Weights and 2-ME and 2-MAA Tissue Partition Coefficients (PCs)

Four GD 13 rats were weighed and asphyxiated with carbon dioxide. Maternal blood and various tissue samples including placentas and embryos were collected for determination of PCs. All major organs were trimmed of adipose tissue, blotted, and weighed. Fat deposits, major muscles, and remaining tissues including bones with remnants of muscle and pelt were pooled and weighed. Tissue PCs were determined as described by Clarke *et al.* (1993). Briefly, 2-ME tissue/air PCs for maternal blood, liver, muscle, gastrointestinal tract, skin, fat, placenta, EAF, and embryos were determined using a vial equilibration - gas chromatography technique designed to determine PCs of the semi-volatile 2-ME. Since 2-MAA is non-volatile, tissue/saline PCs for maternal blood, liver, placenta, embryo, and EAF were determined with tissue homogenates containing 2-MAA spiked with 2-MAA (1-¹⁴C) using an ultracentrifugation technique (Jepson *et al.*, 1994). Tissue/blood PCs were estimated by dividing tissue/saline PCs by the blood/saline PC.

Administration of Chemical and Sample Processing

2-ME mixed with physiological saline was administered between 0800-1100 hours on GD 13 or GD 15 by bolus gavage or by injection into the lateral tail vein with a 26-gauge hypodermic needle attached to a tuberculin syringe. Animals received a constant volume of 2.0 mL/kg such that it contained 15-18 μCi [^{14}C]2-ME per rat at a total dose of 3.3 mmol 2-ME/kg body weight (250 mg/kg). The 2-MAA solution was prepared by neutralization with sodium bicarbonate (Welsch *et al.*, 1987) and injected *i.v.* (2.0 mL/kg) on GD 13 such that the animals received an equimolar amount of 3.3 mmol (298 mg/kg) or 0.067 mmol 2-MAA/kg (5.96 mg/kg) and 9-20 μCi of 1- ^{14}C 2-MAA per animal. Maternal plasma, pooled EAF, and homogenate supernatants from all embryos were collected at various time points from 5 min after injection up to 48 hr. All biological materials were prepared for HPLC analysis by deproteinization using an MPS-1 Micropartition System (Amicon Corporation, Danvers, MA) (Clarke *et al.*, 1991). Samples from 2-ME dosed animals were injected onto an Aminex HPX-87H column (300 x 7.8 mm, Bio-Rad Laboratories, Richmond, CA) which was connected to a Kratos Spectroflow 400 HPLC pump (ABI Analytical Division, Columbia, MD). A mixture of 7.5% acetonitrile in 0.01 N H_2SO_4 was used to separate 2-ME, 2-MAA, ethylene glycol (EG) and an unidentified EG metabolite (Clarke *et al.*, 1993). Samples from 2-MAA injected animals were analyzed on a 15-cm PRP X300 column (Hamilton, Reno, NV). Radioactive peaks from both columns were quantified with a Flow-one Beta radioactivity detector and software (Radiomatic Inst. & Chem. Co., Tampa, FL).

PBPK Model

The present PBPK model for 2-ME/2-MAA builds on that first developed by Clarke *et al.* (1993) and expanded by Terry *et al.* (1995). The revised version is designed to simulate the pharmacokinetic data collected for 2-ME and 2-MAA in the pregnant rat (Fig. 1). 2-ME disposition and elimination are described by flow-limited transfer between maternal

plasma and body tissues and by the rate constants of 2-ME metabolism that quantitate the formation of 2-MAA and EG in the maternal liver. The disposition and elimination of 2-MAA are described by flow-limited transfer between maternal plasma and body tissues and the first-order elimination of 2-MAA from the venous blood compartment. The placenta and embryos are lumped with the other richly perfused tissues in the dam. The mammary glands are considered part of the fat compartment. Values for the placenta, mammary, fat and embryo tissue volumes and tissue perfusions were based on values reported by Fisher *et al.* (1989) in a PBPK model developed to describe trichloroethylene disposition in pregnant rats of the Fischer F344 strain. The model accounted for a rise in maternal body weight throughout pregnancy. Other compartments which were varied with time (increased in size) corresponding to the advance in gestation included the placenta and embryos (lumped into the richly perfused compartment) and the mammary compartment (lumped into the fat compartment).

All PBPK programming, modeling, simulations, and parameter estimations were performed using the SimuSolv modeling and simulation software package (Dow Chemical Company, Midland, MI). The table function capability in SimuSolv was used to interpolate values for body weight, fetal, mammary glands and placenta weights during model simulations.

Kinetic Constants for 2-ME Metabolism

2-ME is metabolized to 2-MAA and to EG. Green *et al.* (1996) determined rate constants for the metabolism of 2-ME to 2-MAA in rat and human hepatocytes based on the production of 2-MAA (the maximum rate, $V_{max} = 1511 \text{ nmol/hr}/10^6$ hepatocytes; and the Michaelis-Menten constant, $K_m = 6.3 \text{ mM}$, see Table 2 in Green *et al.*, 1996). As a means of simplification and due to the low affinity nature of the enzyme reaction (*e.g.*,

K_m in the mM range), a first-order rate constant was estimated by determining the V_{max}/K_m ratio and assuming that there are 128×10^6 hepatocytes/g rat liver (Seglen, 1976). This rate constant was calculated to be 31 L/hr/kg liver and was also used to estimate the rate of production of EG. It was assumed that the metabolism of 2-ME to EG was also first-order at relevant exposure concentrations of 2-ME and that the rate constant was proportional to the ratio of EG/2-MAA formed (see Table 1, Green *et al.*, 1996). The average ratio for the lowest three 2-ME concentration was 0.18, and the first-order constant describing the metabolism of 2-ME to EG was 5.6 L/hr/kg liver (e.g., 31 L/h/kg liver * 0.18).

Kinetic Constant for 2-MAA Elimination

The first-order rate of elimination of 2-MAA from blood (k_e ; hr^{-1}) was optimized by fitting the PBPK model predicted concentration-time curve for 2-MAA in maternal plasma following an i.v. injection of 2-MAA (0.067 mmol/kg) to the respective data.

RESULTS

Tissue Weights and Partition Coefficients

Rat tissue volumes as a function of dam body weight and partition coefficients (PCs) for 2-ME and 2-MAA are listed in Table 1. The collection of tissues for the determination of PCs accounted for approximately 80% of the dams' body weight. Therefore, the maternal tissue volumes used in the present PBPK model were those reported by Andersen *et al.* (1991) and the tissue volumes associated with pregnancy (i.e., placenta, mammary and fat) were those reported by Fisher *et al.* (1989). The measured maternal and fetal body weights were used without modification. The rat blood/air 2-ME PC was 33000, very similar to that reported for mice (34900 Clarke *et al.*, 1993) and humans (32,800,

Johanson and Dynesius, 1988). Tissue/blood PCs for 2-ME were similar to those obtained for mice, *i.e.*, they were within $96 \pm 16\%$ of the values reported in Clarke *et al.*, (1993). As expected for a hydrophilic compound the lowest PC determined was for fat. The 2-ME tissue:blood PCs were near 1.0 for all other tissues evaluated. The lumped richly perfused compartment (including placenta and embryos) was assumed to have a tissue:blood PC equal to 1.1 (arithmetic average of liver, placenta, embryo). All 2-MAA PCs determined were about the same as those of blood whose saline partition coefficient was 1.1. These values agreed closely with those measured in the mouse (Clarke *et al.*, 1993).

2-MAA Administration

When a single dose of 2-MAA (3.3 mmol/kg) was administered on GD 13 by bolus *i.v.* injection, the maternal plasma exhibited a biphasic elimination profile with a terminal elimination half-life of 20 ± 5 hr (Fig. 2a). This pattern was used to optimize the values for the first-order elimination of 2-MAA (k_{ex}) and the slowly-perfused tissue/blood partition coefficient (SPC). The SPC value was not determined by the ultracentrifugation method and exhibited a high sensitivity towards improving the fits of the model to the plasma 2-MAA concentrations. Therefore, the SPC value was adjusted to achieve optimal fits of the model to the 2-MAA maternal plasma concentrations. The optimized value for SPC resulted in consistent simulations in agreement with the measured 2-MAA embryo concentrations following 3.3 mmol/kg *i.v.* (see Figure 2a), as well as at a much lower dose (6.6 μ mol/kg *i.v.*) for both maternal plasma and embryo 2-MAA concentrations (Figure 2b). In addition, a ratio (= 0.68) of the measured 2-MAA fat/blood partition coefficient to the optimized value for SPC is comparable to the ratio value of 0.59 derived from the measured values for 2-butoxyacetic acid (Corley *et al.*, 1994). The success of

the simulations in comparisons with the biological data described above add to our confidence in the optimized SPC value used in the model described here.

2-ME Administration

When a single dose of 2-ME (3.3 mmol/kg or 6.6 μ mol/kg) was administered on GD 13 by bolus i.v. injection, the maternal plasma exhibited a terminal 2-MAA elimination half-life for 20 ± 3 hr. The present model was consistent with the measured plasma for low dose

2-ME (Figure 3a) and the plasma and embryo 2-MAA concentrations following the 3.3 mmol/kg dose (Figure 3b). 2-MAA tissue and plasma concentrations were not determined beyond one hour after administration in the low 2-ME dose experiments (simulations not shown).

The pharmacokinetics of 2-ME in maternal plasma following a gavage dose of 2-ME (3.3 mmol/kg) administered on GD 13 was used to set the value for the first-order oral absorption rate constant (k_a). A k_a value of 3.0 (1/hr) provided reasonable fits to the venous 2-ME (Figure 4a) and 2-MAA (Figure 4b) concentration data. The value for k_a was validated by simulating 2-MAA pharmacokinetics following a single gavage dose of 2-ME (3.3 mmol) administered on GD 15 (Figure 4c) (concentrations of 2-ME in plasma were not determined). (I don't understand what is being said. 2-ME plasma concentrations were determined see attached note)

Discussion

The developmental toxicity and teratogenicity of 2-ME have been ~~in~~ studied in several animal species including mice and rats. A substantial pharmacokinetic data

base collected in mice became the foundation for the validation of a PBPK model applicable to mice (Clarke *et al.*, 1993; Terry *et al.*, 1995). Model extrapolation with validation based on biological data from another species was needed to enhance our trust in the validity of this approach and to proceed with more confidence towards extrapolations for pregnant women. The non-physiological compartment descriptions of 2-MAA disposition used by Clarke *et al.* (1993) and Terry *et al.* (1995) are not readily extrapolated to pregnant women, especially since validation studies cannot be conducted in pregnant women. Therefore, the simulation studies described here provide a physiologically-based model of 2-ME and 2-MAA pharmacokinetics in pregnant rats that will allow more confident predictions of 2-MAA pharmacokinetics in pregnant women following low level 2-ME exposures. The feasibility of this approach has already been demonstrated by pilot extrapolations (Welsch *et al.*, 1995).

The dose-response characteristics and developmental phase specificity of 2-ME's embryotoxic actions (Clarke *et al.*, 1996) resemble closely those in mice (Horton *et al.*, 1985). Thus, rat teratogenicity observations can now be correlated with the rat pharmacokinetic measurements described in this communication and obtained after i.v. or oral bolus doses of 2-ME or i.v. bolus injections of 2-MAA. The plasma elimination half-lives for 2-MAA in rats (19-25 hr) were significantly longer than those determined in mice (5-7 hr) regardless of route of administration or gestational age. This species difference in pharmacokinetics caused significantly larger maternal plasma AUC values in rats than in mice. In contrast, rat maternal plasma C_{max} values were slightly higher when 2-ME was administered by i.v. bolus (6.7 ± 0.1 μ M vs. 5.8 ± 0.4 mM in mice). However, the values in the two species were not different from one another when 2-ME was given by gavage (rat 5.0 ± 0.2 mM vs. mouse 4.8 ± 0.6 mM (Clarke *et al.*, 1993)). The embryo dosimetry-teratogenicity-relationship data suggest that 2-ME-induced digit malformations are better correlated to maternal plasma and embryo AUC values (Clarke

et al., 1993) than to C_{max} . Pregnant rats have a much longer 2-MAA elimination half-life and correspondingly a more extended embryo exposure to 2-MAA. On GD13, structural defects in limbs occurred in 82.5% and 100% of the rat fetuses from dams dosed with 3.3 mmol 2-ME/kg by i.v. injection or gavage, respectively (Sleet *et al.*, 1996). However, in mice 32% and 72% of the fetuses had digit malformations when dams were injected i.v. with 3.3 mmol 2-ME/kg (unpublished observations) or by oral bolus intubation, respectively (Clarke *et al.*, 1992). **The greater integrated area of exposure to 2-MAA (area under the curve) in plots of exposure concentrations against time in rats leads to more toxicity to the developing embryos. Thus in a given species the i.v. route of administration of 2-ME seems to cause less developmental toxicity than gavage dosing (Sleet *et al.*, 1996).**

As on GD 11 in mice (Clarke *et al.*, 1991, 1992), both the AUC and C_{max} values on GD 13 were slightly higher in rat embryos on GD 13 than in maternal plasma. On those gestational days, embryos of these two species are at comparable stages of differentiation, and 2-ME treatment results in a high incidence of malformed digits. As in mice on GD13, rat embryo 2-MAA concentrations on GD 15 were no longer significantly different from maternal plasma values following 2-ME administration. **Rat embryos transplacentally exposed to as much as 500 mg 2-ME/kg maternal body weight on GD 15 exhibit no malformations at term, while 100% of those exposed to that dose on GD 13 are affected by digit malformations (Sleet *et al.*, 1996). ~~Therefore, there appears to be some preferential disposition occurring in GD11 mouse and GD13 rat embryos that affects the levels of 2-MAA in the embryo~~ {Not necessarily. The data don't support this. Rather, there appears to be some phase specific sensitivity occurring in the two species at the same developmental critical window} Since there is not any significant differences in the pharmacokinetics/concentration of 2-MAA in the developing fetuses between GD13 and GD15, the differences in susceptibility**

between GD14 and GD15 developing rat fetuses must be due to pharmacodynamic differences. **It** there appears to be a critical window of susceptibility to 2-MAA exposure in mice (GD11) and rats (GD13). ~~It is not known whether this is a passive (paracellular) or active transport process.~~ Regardless, since the concentration profiles of 2-MAA in the embryos and maternal plasma are quantitatively and qualitatively similar, **the maternal plasma concentrations of 2-MAA appear to be a reasonable indicator of and surrogate for embryo exposure to 2-MAA.**

There is always uncertainty how much detail needs to be included in a PBPK model of the disposition of any given toxicant in pregnant animals and humans. **While on the one hand 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. On the other hand, too simplistic models are difficult to extrapolate between species and dose routes. In the present approach a strategy was pursued that made modifications to existing validated 2-ME/2-MAA PBPK models (Clarke *et al.*, 1993; Terry *et al.*, 1995). The objective was enhance the scientific validity through interspecies extrapolations with the immediate goal of predicting 2-ME and 2-MAA pharmacokinetics in humans. The grouping of the conceptus and placenta into the richly perfused compartment appeared justified because the 2-MAA blood:tissue PCs for the liver (1.1), embryo (1.0) and placenta (1.0) were not very different from one another, suggesting there will not be significant preferential sequestration of 2-MAA in the embryo which during pregnancy is the target tissue of greatest concern for 2-MAA toxicity. Furthermore, altogether the 2-MAA concentration profiles in embryos and maternal plasma are not that much different and any subtle differences would likely result from differences in time of tissue collection and experimental variability. The fact that the changes made to the mouse pregnancy-**

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based PBPK model were consistent with the data collected from pregnant rats exposed to either 2-ME or its embryotoxic metabolite 2-MAA justifies grouping embryos and placenta with the richly perfused tissues of the maternal compartment. The present model was able to describe the pharmacokinetics of both 2-ME and 2-MAA while relying on *in vitro* derived metabolic rate constants for the conversion of 2-ME to 2-MAA and EG thereby lending further support to the modifications made here.

The successful kinetic descriptions of 2-ME and 2-MAA disposition in pregnant rats and their conceptuses by the revised PBPK gestation model provide a firm foundation for efforts to extrapolate the model to human pregnancy. The kinetic insights thus obtained should aid in risk assessment and the establishment of exposure levels for pregnant women who are exposed to 2-ME/2-MAA at their workplace.

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**Validation of a Physiologically-Based Pharmacokinetic Model
of Inhaled Ethylene Glycol Monomethyl Ether in the
Pregnant Rat and Extrapolation to People**

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ABSTRACT

Exposures to 2-methoxyethanol (2-ME) have been found to result in teratogenic effects in rodents and non-human primates. The acetic acid metabolite of 2-ME, 2-methoxyacetic acid (2-MAA) is the likely teratogen, and as such, an understanding of the kinetics of 2-MAA is important when assessing the potential risks to people associated with 2-ME exposures. A previously described physiologically-based pharmacokinetic (PBPK) model of 2-ME/2-MAA kinetics for rats exposed via oral or i.v. administration was extended and validated for inhalation exposures. 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), 6 hrs/day, to 2-ME vapor at 10 ppm and 50 ppm. These concentrations correspond to the known no observed effect level (NOEL) and 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 simulate 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 determine equivalent human exposure concentrations based on 2-MAA dose measures (maximum plasma concentration, C_{max} ; and area under the 2-MAA plasma concentration curve, AUC) that correspond to the concentrations measured at the rat NOEL and LOEL exposure concentrations. It was determined that pregnant women exposed for 8 hrs/day, 5 days/wk, for the duration of pregnancy would need to be exposed to 12 ppm or 60 ppm 2-ME to produce 2-MAA plasma concentrations (C_{max} or AUC) equivalent to the rat NOEL (10 ppm) or LOEL (50 ppm), respectively.

INTRODUCTION

Ethylene glycol monomethyl ether (2-methoxyethanol; 2-ME) is a solvent that was used predominantly as an additive to jet fuel. The material does find some other general solvent uses in industrial application, but the manufacturers specifically do not

recommend its use in consumer products. 2-ME is teratogenic in all tested laboratory animals, including nonhuman primates [Nagano, 1981 #214; Hanley, 1984 #167; Scott, 1989 #102]. 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, 1984 #181]. Based on both *in vivo* and *in vitro* evidence, 2-MAA has been identified as the proximate teratogen in all species tested [Miller, 1983 #203; Yonemoto, 1984 #619; Brown, 1984 #329; Ritter, 1985 #558].

Close evaluation of the dosimetry-teratogenicity relationship has provided evidence that the total exposure to 2-MAA (AUC levels), rather than peak 2-MAA concentrations (C_{max}), is the principal determinant of teratogenesis following exposure to 2-ME [Clarke, 1992 #67]. Quantitation of internal dose metrics, under a variety of exposure conditions, is required to most properly assess the risk to people 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 i.v. exposures [Welsch, 1995 #23; Clarke, 1993 #433; Terry, 1995 #345; Hays, submitted #392]. Welsch et al. [Welsch, 1995 #23] 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 at 0.1 ppm (the proposed Permissible Exposure Limit, OSHA, 1993) with plasma concentrations in mice known to result in teratogenesis (plasma concentration > 1 mM). Welsch et al. [Welsch, 1995 #23] determined that occupational exposures at 0.1 ppm resulted in plasma concentration of about 1 μ M, or 1000-fold below that which is known to cause developmental toxicity in laboratory animals. The human model described by Welsch et al. [Welsch, 1995 #23] was not verified with data nor was the fetal subcompartment developed for the mouse amenable for extrapolation to people, limiting the use of this model in risk assessment evaluations under various exposure scenarios. A more recent PBPK model for 2-ME in the rat [Hays, submitted #392] simplified the description of the fetal compartment and is

better suited for extrapolation to people. This model has been verified for oral and i.v. exposures.

There are three objectives to the work reported here: 1) to extend the oral/i.v. pregnant rat model for 2-ME as described in Hays et al. [Hays, submitted #392] to include the inhalation route; 2) further develop the inhalation model for 2-ME in pregnant women; and 3) determine the concentration of 2-ME in air that results in human internal doses of 2-MAA that are equivalent to the internal doses 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. [Hays, submitted #392] was used for the simulations performed and reported in this paper. Briefly, the model for 2-ME was based on procedures reported by Fisher et al. [Fisher, 1989 #393] in a PBPK model that was developed to describe trichloroethylene disposition in the pregnant rat. The basic model includes compartments representing the liver, fat, slowly and richly perfused tissues (Figure 1). However, three additional tissue masses need to be accounted for during pregnancy; (1) mammary tissue, (2) placental tissue, and (3) fetal tissue. A simplifying assumption in this model is 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 plasma concentrations of 2-MAA as well as to predicted concentrations of 2-MAA for the richly perfused tissues following both oral and i.v. doses of 2-ME or 2-MAA [Welsch, 1995 #23; Clarke, 1993 #433; Terry, 1995 #345; Hays, submitted #392]. The 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. 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. The procedures used by Fisher et al. [Fisher, 1989 #393] to describe the changes in mammary, placental and fetal tissue during pregnancy were also used here.

Time Course Data - Rat

Experimental design

The pharmacokinetics of 2-ME were evaluated in pregnant Sprague Dawley (SD) rats following whole body vapor inhalation exposures. The rats were exposed to two concentrations corresponding to the NOEL, 10 ppm, and the LOEL, 50 ppm, for developmental toxicity [Hanley et al., 1984; OSHA, 1993 #350]. The animals were exposed for 6 hrs/day on gestation days 11-15. Subgroups of 4 rats/time period were sacrificed during and after the final exposure for the analysis of 2-ME and 2-MAA in blood and fetal samples (each litter pooled).

Chemicals

2-ME was used as supplied by Union Carbide (Charleston, WV). The purity of the test material reported by the supplier was $\geq 99.8\%$ with a water content of $\leq 0.02\%$. The test material was stored in its original container at room temperature under a nitrogen headspace until used.

Animals

Timed pregnant Sprague-Dawley CrI: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 morbidity and mortality and body weights were determined prior to the first and last day of exposure.

Exposure conditions and chamber monitoring

Animals were exposed five 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 hr 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 $\leq 5\%$ 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 x 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 efficiencies) and extracted with 0.5 ml ethyl acetate. Separation and quantitation was achieved using a 30 m x 0.32 mm i.d. x 0.5 μ m film thickness Stabilwax DA capillary column (Restec, Bellefonte, PA) and a Hewlett Packard 6890 GC with FID. Splitless injections of ~1.5 μ L of extract were made at an injector temperature of 200°C. The initial oven temperature was 70°C for 2.5 min then increased at 20°C/min to 240°C and held for one min. Hydrogen was used as the carrier gas at 18 psi constant pressure. The FID detector temperature was 270°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, Bellefonte, PA) was used to concentrate the sample and provide an initial sample clean up step prior to chromatography. Second, a 25 cm section of deactivated 0.25 mm i.d. 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 µg/g.

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°C until analyzed. Blood samples were collected from four rats/sampling time at 1 and 3 hours during the exposure, immediately post-exposure, and at 0.5, 1, 2, 4, 8, 18, 42, 66 and 90 hours post exposure. 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-hour post-exposure blood collection time point was placed in individual metabolism cages (Lab Products; Seaford, DE) for the 0-18 hr post-exposure urine collection. Each animal was provided access to water but not feed. Urine samples were collected over dry ice and stored at -70°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. As 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 hours post-exposure were analyzed for 2-ME and 2-MAA as described above.

PBPK Model for Pregnant Women

The PBPK model that was used to simulate 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 (Figure 1). As with the rat model, the mammary tissue was grouped with the adipose tissue and the placenta and fetus 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 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 and the kinetic constants of metabolism of 2-ME to 2-MAA and to EG were determined from the data of Green et al. [Green, 1996 #335] (see Table 1). The rate constant describing the urinary excretion of 2-MAA (K_{ex} ; L/hr) was determined by fitting model output to the data from Groeseneken et al. [Groeseneken, 1989 #105]. As described by Hays et al. [Hays, submitted #392], K_{ex} and the slowly perfused tissue:blood partition coefficients were the only model parameters that were adjusted for rats. The only model parameter that had to be "fit" in the human model was K_{ex} . All other parameters were measured or taken from the literature.

Estimating Equivalent Internal Doses

The PBPK model for the rat was used to simulate the expected peak (C_{max}) and average area under the plasma concentration curve (AUC) for 2-MAA in the rat following 10 ppm and 50 ppm 2-ME exposures for 8 hours. These same values for C_{max} and AUC in the rat were used as the target values for simulations using the human pregnancy model. Various inhaled concentrations of 2-ME for 8-hour periods were used as inputs to the human model until C_{max} and AUC values for 2-MAA in plasma were equal to those determined for rats. The resulting inhaled 2-ME concentrations for people were then

considered to be equivalent to the NOEL and LOEL in rats for developmental effects, based on these internal dose metrics.

All model simulations were performed using the Advanced Continuous Simulation Language (ACSL, MGA Associates, Inc., Cambridge, Massachusetts).

RESULTS

Animal Experiments

The average concentrations of 2-ME in the chambers determined by GC/FID were 10.7 and 47.2 ppm for the target concentrations of 10 and 50 ppm. The 50 ppm chamber exposures were more variable than expected with daily concentrations ranging from 34.5 ppm (3rd exposure) to 59.2 ppm (5th 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.

Simulation of Rat Data

Since the half-life of 2-MAA in urine is on the order of 48 hours in rats [Medinsky, 1990 #523; Cheever, 1988 #330], exposures to 2-ME were conducted over a five-day period to allow near steady-state conditions to be achieved. Preliminary modeling indicated that five exposures of 6 hrs/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 five 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 the two exposure groups are provided in 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.

Peak blood concentrations of 0.2 mg/L (Figure 2A) and 1.3 mg/L (Figure 3A) 2-ME were reached at the end of the final exposures on GD 15 for the 10 ppm and 50 ppm exposures, respectively. The model simulations 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 mg/L (Figure 2B) and 62.7 mg/L (Figure 3B) were reached at the end of the 10 ppm 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 8 hr post-exposure. 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 hr post-exposure (Figures 2B and 3B).

Simulation of Human Data

Groeseneken et al. [Groeseneken, 1989 #105] determined the pulmonary retention of 2-ME and the rate of 2-MAA excretion in urine from male human volunteers exposed to 16

mg/m³ (5 ppm) of 2-ME. The exposure regimen consisted of four 50-minute exposures with a 10-minute break at the end of each 50-minute exposure for urine collection, with urine collection continuing for five days. The human PBPK model described above (less the parameters used to describe pregnancy) was used to simulate the urinary excretion rate data collected by Groeseneken et al. for male volunteers [Groeseneken, 1989 #105]. The first-order urinary excretion rate constant (K_{ex}) was allowed to vary until a reasonable fit was achieved (Figure 4). All other model parameters remained fixed for this simulation. The PBPK model predicted 15.6 mg of 2-MAA should be excreted during the 120 hours of the exposure and post-exposure periods described by Groeseneken et al. [Groeseneken, 1989 #105]. The total 2-MAA excreted as reported by Groeseneken et al. [Groeseneken, 1989 #105] was 16.6 mg. These simulations allowed for reasonable estimation of the final parameter, K_{ex} , needed for human model development.

Equivalent Internal Dosimetry

The PBPK model for the pregnant rat was used to determine the blood C_{max} and average 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 and #b). The model was then used to predict C_{max} and 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 ppm and 50 ppm, respectively. The human pregnancy model was used to predict these same internal dosimeters for pregnant women exposed for 8 hrs/day, 5 days/wk for 270 days at various inhaled concentrations of 2-ME. The human equivalent inhaled concentrations to the NOEL in the rat (10 ppm), was determined to be 12 ppm using either C_{max} or 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 AUC for 2-MAA.

DISCUSSION

The model described in this work produces reasonable simulations of 2-ME and 2-MAA kinetics in pregnant rats following multi-day exposures to 2-ME. There are, however, some differences between model predictions and observed data. The model slightly over predicts (about a factor of 2) the 2-ME maternal plasma concentrations for rats (Figures 2A and 3A) while still producing good fits to the 2-MAA maternal plasma concentrations (Figures 2B and 3B). The over predictions of 2-ME in maternal rat plasma might be due to 1) an over prediction of the amount of 2-ME absorbed via inhalation, 2) an under prediction 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 simulations were found to be more consistent with the observed maternal plasma concentrations of 2-ME in rats if lower values of the alveolar absorption fraction (K_{alv}) is used, such as a value of 0.6 as was reported by Johanson et al. [Johanson, 1986 #291] for a related glycol ether, 2-butoxyethanol (simulation not shown). However, this would yield predictions of the maternal 2-MAA concentrations in rat plasma that would slightly under-estimate the experimental results. In addition, it was felt prudent to use the alveolar absorption fraction measured by Groeseneken et al. [Groeseneken, 1989 #105] for people since it was an experimentally derived value. The relevance of this value for rats is still not resolved.

The model described in this paper used metabolic parameters determined in vitro by Green et al. [Green, 1996 #335]. The model fits to maternal plasma concentrations of 2-ME could be improved by assuming a larger percentage of 2-ME metabolism resulted in the production of 2-MAA versus EG. However, the model used as the basis for this work [Hays, submitted #392] that included the rate constants of metabolism that were used here was accurate in predicting maternal plasma concentrations of 2-MAA in rats following 2-ME i.v. and oral administrations. Therefore, the values determined by Green et al. [Green, 1996 #335] were used in these simulations 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. [Kawamoto, 1990 #83] found that 2-ME induced alcohol dehydrogenase (ADH) activities in hepatic cytosols from rats pretreated with 2-ME (100 and 300 mg/kg body weight) 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 percent over rats exposed to vehicle control [Kawamoto, 1990 #83], 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 simulations performed by 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 i.v. doses of 2-ME [Hays, submitted #392] 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 simulations 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 five-day period. The ability of a PBPK model to be within a factor of two after five days of exposure is excellent 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 multi-day exposure studies as was described here.

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, 1989 #105] and that data was used to set one of the model parameters. It is reassuring, however, that parameters 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 simulation 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 simulations. 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 percent higher than exposure concentrations corresponding to the rat NOEL and LOEL. Welsch et al. [Welsch, 1995 #23] reported that maternal plasma concentrations of 2-MAA >1 mM in pregnant mice results in teratogenic effects. 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. [Welsch, 1995 #23]. The work reported here indicates that pregnant women exposed to inhaled concentrations of 2-ME for 8 hrs/day, 5 days/wk for the duration of pregnancy do not reach blood 2-MAA concentrations that are known to be teratogenic in mice and rats until the exposure concentration reaches 60 ppm or higher.

The use of PBPK models in chemical risk assessments designed to aid in developing regulatory standards has not yet gained widespread acceptance [Hays, 1998 #394]. Combining information regarding the mechanisms of toxic action for a chemical with more biologically pertinent measures of dose allows added confidence in the extrapolations needed for risk assessments. It is hoped that approaches as described in this paper will help reduce the uncertainties present in all risk assessments so the

regulatory standards that are established might be based on the best information that the state-of-the-science can provide. While it is not feasible to eliminate all uncertainties in risk assessment, the results presented here can serve as the basis for a more biologically-realistic assessment of the teratogenic hazards posed by exposure of pregnant women to 2-ME.

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{References not in EndNote glycol ethers file and/or only in Excel table: they will need to be formatted to journal style and inserted in appropriate order, or added to the EndNote file.}

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Figure Legends

Figure 1: The structure of the PBPK model describing 2-ME and 2-MAA kinetics in rats and people.

Figure 2: 2-ME (Figure 2A) and 2-MAA (Figure 2B) venous blood concentrations during and following a 6 hr exposure to 2-ME at 10 ppm on GD 15. Values are the mean \pm standard deviation (SD) of up to 4 rats (or the fetuses from 4 rats)/sample time. The smooth curves are those predicted by the PBPK model.

Figure 3: 2-ME (Figure 3A) and 2-MAA (Figure 3B) venous blood concentrations during and following a 6 hr exposure to 2-ME at 50 ppm on GD 15. Values are the mean \pm SD of up to 4 rats (or the fetuses from 4 rats)/sample time. The smooth curves are those predicted by the PBPK model.

Figure 4: The rate of urinary excretion of 2-MAA from human volunteers exposed to 16 mg 2-ME/m³ (see text for experimental details). The smooth curve was produced by the human PBPK model by adjusting K_{ex} until a reasonable fit was achieved.

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**Development of a Physiologically-Based Pharmacokinetic Model of
Ethylene Glycol Ethyl Ether Acetate and
Ethoxy Acetic Acid Kinetics in Rats and People**

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ABSTRACT

The solvents ethylene glycol monoethyl ether acetate (EGEEA) and ethylene glycol monoethyl ether (EGEE) are known to be rodent teratogens, exerting their toxic effects through the action of their metabolite ethoxyacetic acid (EAA). Thus risks associated with exposure to these compounds are best evaluated based on a measure of EAA dosimetry. The goals of the work reported here were to develop physiologically-based pharmacokinetic (PBPK) models of EGEEA and EGEE for pregnant rats and people and apply these models to estimate human exposure levels equivalent to rodent no observed effect level (NOEL) and lowest observed effect level (LOEL) for developmental effects, in terms of internal EAA dosimetry. Pregnant Sprague-Dawley rats were exposed to concentrations of EGEEA corresponding to the NOEL and LOEL of this compound. Maternal blood and urine and fetal tissue concentrations of EGEE and EAA measured in these experiments were used to validate the rat EGEEA and EGEE models. Data collected by other researchers were used to validate the human EGEEA and EGEE models. The models were considered reliable for estimating internal concentrations of EAA at the NOEL and LOEL, based on fits achieved for the blood, urine, and fetal tissue data. The human equivalent inhaled concentration to the rat NOEL for EGEEA (50 ppm) was determined to be 25 ppm using the maternal blood area under the curve (AUC) and 40 ppm using the maximum concentration achieved in maternal blood (C_{max}). The human equivalent inhaled concentration to the rat LOEL for EGEEA (100 ppm) was determined to be 55 ppm using the maternal blood AUC and 80 ppm using the maternal blood C_{max} .

INTRODUCTION

Ethylene glycol monoethylether (EGEE) and ethylene glycol monoethyl ether acetate (EGEEA) have been used in a variety of solvent applications. EGEE has been used as a solvent for nitrocellulose and for natural and synthetic resins, as well as a component in lacquers and varnish removers. EGEEA has been used as a blush retardant in lacquers, as a solvent for nitrocellulose, oils, and resin, in wood stains, varnish removers, and in

products for the treatment of textiles and leathers. Occupational exposures to EGEE and EGEEA are primarily via inhalation (OSHA, 1993), but the glycol ethers are also known to be readily absorbed dermally (Guest, et al., 1984; Sabourin, et al., 1992; Kezic, et al., 1997).

EGEE and EGEEA are known to be teratogenic and fetotoxic in rodents (Hardin, et al., 1982; Andrew and Hardin, 1984; Doe, 1984; Nelson, et al., 1984; Kalf, et al., 1987, Union Carbide, 1984), produce testicular toxicity in male rodents (Cheever, et al., 1984; Foster, et al., 1984; Kalf, et al., 1987), and also produce hematological effects (Barbee, et al., 1984; Nagano, 1979; Union Carbide, 1984). The acid metabolite, ethoxy acetic acid (EAA), resulting from metabolism of both EGEE and EGEEA, has been identified as the toxic form of these chemicals (Cheever, et al., 1984; Foster, et al., 1984; Groeseneken, et al., 1988). Therefore, assessment of potential risks posed by exposure to EGEE or EGEEA should include an evaluation involving measures of EAA dosimetry.

The distribution, metabolism, and elimination of EGEEA following pulmonary uptake are the same as for EGEE. Once inhaled, EGEEA is rapidly converted to EGEE via carboxylesterases in pulmonary mucosa (Stott and McKenna, 1985), and by plasma esterases (Guest, et al., 1984; Groeseneken, et al., 1987a, b). The conversion is so rapid that very little, if any, EGEEA is distributed to the tissues. EGEE is metabolized via alcohol dehydrogenase and aldehyde dehydrogenase to form EAA and via microsomal oxidation to form ethylene glycol (EG). EAA is further metabolized to form a glycine conjugate in rodents (N-ethoxyacetyl glycine; EAG) (Kennedy, et al., 1993), however, the glycine conjugate of EAA has not been reported in humans (Groeseneken, et al., 1988). Both EG and EAA undergo further metabolism to form carbon dioxide. The major metabolites excreted in the urine following an exposure to EGEE are EAA, EG and EAG (Kennedy, et al., 1993).

Physiologically-based pharmacokinetic (PBPK) models describing the kinetics of several glycol ethers in rodents and people are available. Models for ethylene glycol

monomethyl ether (EGME) have been described for mice (Clarke, et al., 1993; Terry, et al., 1995; Welsch, et al., 1995), rats (Hays, et al., 1999a, b), and people (Welsch, et al., 1995; Hays, et al., 1999b). Welsch et al. (1995) and Hays et al. (1999b) have used these models to predict acid metabolite concentrations in people for comparison to known teratogenic plasma acid levels in mice and rats. No such models currently exist for EGEE or EGEEA. Biologically-based risk assessments for these compounds must account for the production, distribution, and elimination of EAA, all processes well suited for evaluation and prediction by pharmacokinetic modeling.

The objectives of the work described here are to develop PBPK models for EGEEAms pregnant rats and people and to use those models to estimate human exposures that are equivalent, based on internal EAA dosimetry, to rat exposures resulting in a no observed effect level (NOEL) and a lowest observed effect level (LOEL). These models are intended to be used in further evaluations to determine safe exposure concentrations in occupational and environmental settings.

METHODS

PBPK Model - Rat

The PBPK model developed for EGME kinetics in rats (Hays, et al., 1999a) was used as the basis for the EGEEA/EGEE/EAA model used in this paper. Briefly, the same model structure was used and contained three submodels (one for EGEEA, EGEE and EAA), each with discrete compartments for the lung, liver, fat and richly and slowly perfused compartments (Figure 1). For simplifying purposes, the growing fetuses were grouped into the richly perfused tissue compartment based on the observation that rat maternal plasma and rat fetal tissue concentrations of MAA were essentially identical following i.v. and oral exposures to EGME (Clarke, et al., 1993; Welsch, et al., 1995). The rat tissue:blood partition coefficients for EGEE and EAA were assumed to be the same as for EGME and MAA and were taken directly from Hays et al. (Hays, et al., 1999a) (see

Table 1). The first-order rate constants describing the metabolism of EGEE to EAA and EGEE to EG were derived from Green et al. (Green, et al., 1996) and adjusted for differences between rate constants in rat hepatocyte suspensions and in vivo for ethylene glycol monobutyl ether (Corley, et al., 1994) (see Table 1). Time varying body weights (due to pregnancy) were also included in the model description. The blood concentration time-course data for EGEE and EAA collected during and post-inhalation exposure (as described below) were used to test the ability of the model to predict these concentrations as a means of model verification.

Rat Data

Experimental design

The pharmacokinetics of EGEEA were evaluated in pregnant Sprague-Dawley rats following whole body vapor inhalation exposures. The rats were exposed to two concentrations of EGEEA corresponding to the no observed effect level (NOEL), 50 ppm (Doe, 1984; OSHA, 1993), and lowest observed effect level (LOEL), 100 ppm (OSHA, 1993, Union Carbide, 1984) for developmental toxicity. The animals were exposed for 6 hr/d for five consecutive days on gestation days 11-15. Subgroups of 4 rats/time period were sacrificed during and after the final exposure for the analysis of EGEEA and EAA in blood, urine and fetal samples (each litter pooled).

Due to the extremely rapid hydrolysis of the acetate moieties of glycol ethers (Stott and McKenna, 1985; Groeseneken, et al., 1987), analytical methods were optimized for the quantitation of the parent glycol ether, EGEE, and its metabolite, EEA, in all blood, urine and fetal samples collected from animals exposed to EGEEA. Extracts from the blood samples collected from the highest EGEEA exposure group (100 ppm) were analyzed for the presence of EGEEA using the same GC method described below for EGEE and EAA.

Chemicals

EGEEA was used as supplied by Union Carbide (Charleston, WV). The purity of each test material reported by the supplier was $\geq 99.8\%$ with a water content of $\leq 0.02\%$. The test material was stored in its original container at room temperature under a nitrogen headspace until used.

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 five consecutive days (gestation days 11-15) to targeted concentrations of 50 and 100 ppm EGEEA in stainless steel and glass Hazelton 2000 inhalation chambers. Total daily exposure durations were 6 hr plus the time to reach 90% of the target concentration (T_{90}). T_{90} 's averaged 12 min for EGEEA. 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 EGEEA in each chamber was controlled by adjusting the test material pump rate and dilution airflow. The distribution of material over 12 sampling sites within each chamber was verified to be $\leq 5\%$ 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 x 0.53 mm ID, RTX-5, 5 μ m 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 EGEEA was 1.6 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 EGEEA. 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 EGEEA the largest relative standard deviation observed for successive readings at a single port was 0.61%.

Analytical methods for rat blood, urine, and fetuses

An analytical method was developed to simultaneously quantitate EGEE and EAA 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 0.1 M sulfuric acid, addition of 0.25 g sodium sulfate (to improve extraction efficiencies) and extracted with 0.5 ml ethyl acetate. Separation and quantitation was achieved using a 30 m x 0.32 mm id x 0.5 µm film thickness Stabilwax DA capillary column (Restek, Bellefonte, PA) and a Hewlett Packard 6890 GC/FID. Splitless injections of ~1.5 µL of extract were made at an injector temperature of 200°C. The initial oven temperature was 70°C for 2.5 min then

increased at 20°C/min to 240°C and held for one min. Hydrogen was used as the carrier gas at 18 psi constant pressure. The FID detector temperature was 270°C.

To achieve the sensitivity and selectivity needed to simultaneously analyze for EGEE and EAA, two modifications to the standard capillary GC/FID system were required. First, a Cyclo double gooseneck injection liner (Restek, Bellefonte, PA) was used to concentrate the sample and provide an initial sample clean up step prior to chromatography. Second, a 25-cm section of deactivated 0.25 mmid capillary column was connected to the Stabilwax-DA column prior to the detector to improve peak shape for quantitation. Methoxyethanol and methoxyacetic acid were used as internal standards for EGEE and EAA acid analyses. All standard curves were generated using matrix-spiked standards to correct for extraction efficiencies. Detection limits for the glycol ether and alkoxyacetic acid metabolite were approximately 0.012 µg/g.

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°C until analyzed. For EGEEA, blood samples were collected from four rats/sampling time at 1 and 3 hr during the exposure, immediately post-exposure and at 0.5, 1, 2, 4, 8, 18, 30, and 42 hr post-exposure. Blood samples were collected from control animals, spiked with known quantities of EGEE and EAA 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 EGEE and EAA by gas chromatography/flame ionization detection.

Urine sampling and analyses

Each rat scheduled for the 18-hour post-exposure blood collection time point was placed in individual metabolism cages (Lab Products; Seaford, DE) for the 0-18 hr post-exposure urine collection. Each animal was provided access to water but not feed. Urine

samples were collected over dry ice and stored at -70°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. As with blood, 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 hr post-exposure were analyzed for EGEE and EAA as described above.

PBPK Model – Human

The human pregnancy model for EGME described by Hays et al. (1999 b) was used as the basis of the model developed here. All physiological and anatomical parameters, including those that change due to pregnancy, were as described by Hays et al. (1999b). The human blood:air partition coefficients for EGEE were from Johanson and Dynesius (1988) with all other tissue:blood coefficients set equal to those for EGME in rat tissues. Johanson and Dynesius (1988) were not able to measure a blood:air coefficient for EGEEA due to the rapid hydrolysis of EGEEA to EGEE by blood esterases. However, since the saline:air coefficients for EGME and EGEE (35869 and 23069, respectively) were very similar to the blood:air for EGME and EGEE (32836 and 22093, respectively), we assumed the saline:air value for EGEEA (Johanson and Dynesius, 1988) was a suitable surrogate for a blood:air partition coefficient. The tissue:blood partition coefficients for EAA were set equal to the MAA partition coefficients used for rats (Hays, et al., 1999a). The data of Green et al. (1996) were used to determine the rate of metabolic conversion of EGE to EAA and to EG. This rate was adjusted for differences between rate constants in rat hepatocyte suspensions and in vivo for ethylene glycol monobutyl ether (Corley, et al., 1994). The rapid metabolism of EGEEA to EGEE by

blood esterases was assumed to essentially occur instantaneously. The rate of elimination of EAA in the urine (K_e ; hr^{-1}) was estimated by comparing model simulations to the data from Groeseneken et al. (1986b).

Verification of the human PBPK model for EGEE and EGEEA kinetics was performed by comparing model output to various data from Groeseneken et al. (1986a, 1986b; 1987a, 1987b). For these experiments male human volunteers were exposed to 10, 20, or 40 mg EGEE/ m^3 or 14, 28, or 50 mg EGEEA/ m^3 for four hours with a 50 minute on, 10 minute off exposure regimen for each hour. A one-way breathing valve was used which allowed measurements of inhaled and exhaled concentrations of EGEE (Groeseneken, et al., 1986a) or EGEEA (Groeseneken, et al., 1987b). Urine was collected for 48 hours post-exposure and EAA concentrations were determined following EGEE (Groeseneken, et al., 1986b) or EGEEA (Groeseneken, et al., 1987a) exposures.

Estimating Equivalent Internal Doses

The PBPK model for the rat was used to simulate the expected peak (C_{max}) and average area under the blood concentration curve (AUC) for EAA in the rat following 50 ppm and 100 ppm EGEEA exposures for 8 hours. These same values for C_{max} and AUC in the rat were used as the target values for simulations using the human pregnancy model. Various inhaled concentrations of EGEEA for 8-hour periods were used as inputs to the human model until C_{max} and AUC values for EAA in blood were determined to be equal to those determined for rats. The resulting inhaled EGEEA concentrations for people were then considered to be equivalent to the NOEL and LOEL in rats for developmental effects, based on these internal dose metrics.

All model simulations were performed using the Advanced Continuous Simulation Language (ACSL, MGA Associates, Inc., Cambridge, Massachusetts).

RESULTS

Animal Experiments

The average concentrations of EGEEA in the chambers were 47.5 and 108.2 ppm for the target concentrations of 50 and 100 ppm, respectively. The average daily chamber concentrations were within $\pm 10\%$ of target. The temperature and relative humidity of the chamber during each experiment averaged 101% and 81-82% 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.

Simulation of Rat Data

Since the half-life of EAA in urine is on the order of 48 hours in rats (Cheever, et al., 1984; Medinsky, et al., 1990), exposures to EGEEA were conducted over a five-day period to allow near steady-state conditions to be achieved. Preliminary modeling indicated that five exposures of 6 h/d should be sufficient. An array of the measured exposure concentrations was used in the model to allow for different exposure concentrations on each of the exposure days (since the actual concentrations of EGEEA 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 (after the five days of exposure) were used to derive a linear regression line describing the body weight of each exposure group (i.e., 50 and 100 ppm exposed animals). The resulting regression lines for the two exposure groups are provided in Table 1. The regression line was used to derive an array of body weights as a function of time for modeling EGEEA and EAA concentration time-courses in blood. The TABLE function of ACSL was used to interpolate and extrapolate body weights at all times of the simulation.

No EGEEA was detected in any rat blood sample following exposure to the highest concentration (100 ppm). The parent glycol ether formed by the hydrolysis of the acetate, EGEE, was detected in the blood of rats at all sampling times during exposure. Although there was some variability in the concentration, steady-state concentrations appear to have been reached during exposure at both concentrations. Peak EGEE blood concentrations of 0.7 mg/L (Figure 2A) and 2.3 mg/L (Figure 3A) were reached at the end of the final exposures on GD 15 for the 50 ppm and 100 ppm exposures, respectively. Model simulations of the rat data compared very well to measured EGEE maternal blood concentrations at both 50 ppm and 100 ppm (Figure 2A and 3A). For the major metabolite, EAA, peak blood concentrations of 24.7 mg/L (Figure 2B) and 73.5 mg/L (Figure 3B) were reached at the end of the 50 ppm and 100 ppm exposures, respectively. The PBPK model provided reasonable predictions of the EAA concentration time-courses for both exposure concentrations in rats.

Concentrations of EGEE in the fetal samples collected at the end of the last exposure were higher (12-36%) than the corresponding maternal blood sample concentrations (data not shown). No EGEE was detected in rat fetuses 8 hr post-exposure. EAA concentrations in rat fetuses averaged 40% higher than the corresponding rat maternal blood concentrations at 0 and 8 hr post-exposure (Figures 2B and 3B).

The model predicts that 2.07 and 4.92 mg EAA will be excreted in the urine after exposure to 50 and 100 ppm EGEEA, respectively. Post-exposure urinary excretion of EAA was measured to be 1.04 mg for the 50 ppm exposures and 2.83 mg for the 100 ppm exposures (no free EGEE or EGEEA was detected in any urine sample). However, the model predictions would be expected to include all EAA-derived urinary metabolites, and the analytical method measures only EAA. Experimental work (Cheever, et al., 1984; Groeseneken, et al., 1988; Sabourin, et al., 1992; Kennedy, et al., 1993) indicates that EAA-derived metabolites such as glycine conjugates may contribute 14 to 74 percent more EAA-derived urinary metabolites. The average in studies with male SD rats, the

strain used in the study reported here, was 68 percent (Cheever, et al., 1984). It is therefore estimated that measured amounts of urinary EAA should be multiplied by a factor of 1.7 to approximate the total flux through the EAA pathway. The resulting estimates of 1.77 and 4.81 mg EAA-derived urinary metabolites are reasonably close to model predictions.

Simulation of Human Data

The first-order rate constant ($K_{e,}$) that describes the urinary elimination of EAA was varied until reasonable fits were achieved to the data following EGEE exposures from Groeseneken et al. (1986b) (see Figure 4; only 20 mg/m³ data shown, 10 and 40 mg/m³ data were fit equally as well). This parameter, as well as all others, were held constant (Table 1) for the remainder of the simulations of human data. A reasonable fit (Figure 5; only 28 mg/m³ data shown, 14 and 50 mg/m³ data were fit equally as well) was also achieved to the urinary excretion of EAA following EGEEA exposures to male human volunteers (Groeseneken, et al., 1987a).

Model simulations of exhaled breath concentrations of EGEE provided good fits to the data of Groeseneken et al. (Groeseneken, et al., 1986) (Figure 6; data and fit shown for 20 mg/m³ exposure only; similar results were obtained for 10 and 40 mg/m³ exposures). The model simulations did predict a more rapid decline of exhaled breath concentrations at the end of exposure than was observed. The exhaled breath concentration data for EGEEA (Groeseneken, et al., 1987) were also well represented during exposure (Figure 7; data and fit shown for 28 mg/m³ exposure only; similar results were obtained for 14 and 50 mg/m³ exposures), but the model significantly over-estimated the rate of decline of exhaled breath concentrations in the post-exposure period. In addition, the model under-predicted EGEE exhaled breath concentrations measured during EGEEA exposures by slightly more than a factor of 10 (data and simulations not shown).

Equivalent Internal Dosimetry

The PBPK model for the pregnant rat was used to determine venous blood C_{max} and average AUC for EAA under the experimental conditions described by Doe (1984) and Union Carbide (1984) that were used in the rat studies described here. The model was considered reliable for estimating these values based on the fits achieved for predicting rat EAA blood and fetus concentrations (Figures 2B and 3B). The human pregnancy model was used to predict these same internal dosimeters for pregnant women exposed for 8 hrs/day, 5 days/wk for 270 days at various inhaled concentrations of EGEEA. The human equivalent inhaled concentrations to the NOEL in the rat (50 ppm), was determined to be 25 ppm using AUC for EAA and 40 ppm using C_{max} . The human equivalent inhaled concentration to the LOEL in the rat (100 ppm), was determined to be 55 ppm using AUC for EAA and 80ppm using C_{max} .

DISCUSSION

The model predictions of the rat data for EGEE and EAA in maternal blood and EAA in fetal tissue after exposure to EGEEA were very good for both inhaled concentrations (Figures 2 and 3). This accomplishment is significant because no adjustments were made to metabolic parameters—the only adjusted parameter was the urinary excretion rate of EAA. It is also noteworthy that the model was predictive of EGEEA disposition after repeated exposure (5 days), given the potential for alteration of metabolism during such an exposure.

The human model predictions were also quite successful. Again, no adjustments were made to any metabolic parameters, and the value for the urinary excretion rate (K_{ex}) developed by fitting urinary excretion of EAA after EGEE exposure (Fig. 4) worked very well at predicting EAA excretion after EGEEA exposure (Fig. 5). Exhaled breath concentration of EGEE during EGEE exposure was accurately modeled; the postexposure breath concentrations were predicted with reasonable accuracy, although the initial rate of

decline was somewhat overpredicted (Fig. 6). Predictions of EGEEA in exhaled breath were good during exposure, but significantly higher than reported postexposure concentrations (Fig. 7). Possible explanations include the difficulty of accurately measuring such low concentrations of EGEEA and the fact that the water:air partition coefficient for EGEEA was used in place of a blood:air partition coefficient. Another possibility is that EGEEA was retained in the mucous membranes of the lungs due to a "wash in-wash out" effect (Gerde and Dahl, 1991; Johanson, 1991; Medinsky, et al., 1993) for water-soluble compounds and the EGEEA observed in postexposure exhaled breath is being desorbed from these mucous membranes. It is important to note, however, that data involving the preserved teratogenic form of the chemical, FAA, were all well fit by both the rodent and human models.

The use of internal dosimeters in risk assessment is preferable to administered dose because the internal measures provide better indicators of toxicity for effects occurring away from the site of entry. So far, however, this approach has had limited application to development of regulatory toxicity criteria (Hays, et al., 1998). The accuracy of the rat and human PBPK models at predicting internal dosimetry of EGEEA metabolites lends a high degree of confidence to use model-estimated internal dosimeters to deduce human-equivalent concentrations for NOEL and LOEL exposures in the rat. Lacking information as to whether blood AUC or peak concentration is a more appropriate dose metric for teratogenic effects of EGEEA in rodents, it would be prudent at this time to consider 25 ppm and 55 ppm as being the human-equivalent NOEL and LOEL concentrations for EGEEA.

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FIGURE LEGENDS

Figure 1. Schematic of the physiologically based pharmacokinetic description for ethylene glycol monoethyl ether acetate (EGEEA), ethylene glycol monoethyl ether (EGEE), and 2-ethoxy acetic acid (EAA) disposition in pregnant rats and humans. EGEEA is metabolized to EGEE in the blood and EGEE is metabolized to EAA and ethylene glycol in the liver. The fetal and placental tissues are grouped with the richly perfused compartment and the mammary tissue is grouped with the fat compartment.

Figure 2. Concentrations in maternal venous blood (◆) and fetal tissue (⊙) during and following exposure of GD-15 pregnant rats to 50 ppm EGEEA. Values are the mean \pm SD of up to 4 rats (or the fetuses from 4 rats)/sample time. The smooth curves are those predicted by the PBPK model. (A) Concentrations of EGEE. (B) Concentrations of EAA.

Figure 3. Concentrations in maternal venous blood (◆) and fetal tissue (⊙) during and following exposure of GD-15 pregnant rats to 100 ppm EGEEA. Values are the mean \pm SD of up to 4 rats (or the fetuses from 4 rats)/sample time. The smooth curves are those predicted by the PBPK model. (A) Concentrations of EGEE. (B) Concentrations of EAA.

Figure 4: The rate of urinary excretion of 2-EAA from human volunteers exposed to 20 mg EGEE/m³ (see text for experimental details). The smooth curve was produced by the human PBPK model by adjusting K_{ex} until a reasonable fit was achieved.

Figure 5: The rate of urinary excretion of 2-EAA from human volunteers exposed to 28 mg EGEEA/m³ (see text for experimental details). The smooth curve was produced by the human PBPK model.

Figure 6: Exhaled breath concentrations of EGEE from human volunteers exposed to 20 mg EGEE/m³ (see text for experimental details).

Figure 7: Exhaled breath concentrations of EGEEA from human volunteers exposed to 28 mg EGEEA/m³ (see text for experimental details). Andrew, F. D. and B. D. Hardin (1984). "Developmental effects after inhalation exposure of gravid rabbits and rats to ethylene glycol monoethyl ether." Environ Health Perspect 57: 13-23