Toxicology and Applied Pharmacology **165,** 63–73 (2000)
doi:10.1006/taap.2000.8927, available online at http://www.idealibrary.com on IDEAL®

A Toxicokinetic Study of Inhaled Ethylene Glycol Ethyl Ether Acetate and Validation of a Physiologically Based Pharmacokinetic Model for Rat and Human

Michael L. Gargas,*[,1] Tipton R. Tyler,† Lisa M. Sweeney,* Richard A. Corley,‡ Karl K. Weitz,‡ Terryl J. Mast,‡ Dennis J. Paustenbach,§ and Sean M. Hays*[,2]

*ChemRisk, A Service of McLaren/Hart, Inc., Cleveland, Ohio; †Union Carbide Corporation, Danbury, Connecticut; ‡Battelle, Pacific Northwest Laboratories, Richland, Washington; and §Exponent, Inc., Menlo Park, California

Received August 23, 1999; accepted March 7, 2000

A Toxicokinetic Study of Inhaled Ethylene Glycol Ethyl Ether Acetate and Validation of a Physiologically Based Pharmacokinetic Model for Rat and Human. Gargas, M. L., Tyler, T. R., Sweeney, L. M., Corley, R. A., Weitz, K. K., Mast, T. J., Paustenbach, D. J., and Hays, S. M. (2000). *Toxicol. Appl. Pharmacol.* **165, 63–73.**

The solvents ethylene glycol monoethyl ether acetate (EGEEA) and ethylene glycol monoethyl ether (EGEE), at sufficiently high doses, are known to be rodent developmental toxicants, exerting their toxic effects through the action of their metabolite 2-ethoxyacetic acid (2-EAA). Thus risks associated with exposure to these compounds are best evaluated based on a measure of the internal dose of 2-EAA. The goals of the work reported here were to develop physiologically based pharmacokinetic (PBPK) models of EGEEA and EGEE for pregnant rats and humans. These models were used to identify human exposure levels (ppm in air) equivalent to the rat no observed effect level (NOEL) and lowest observed effect level (LOEL) for developmental effects (Hanley *et al.,* **1984). We exposed pregnant Sprague–Dawley rats to concentrations of EGEEA corresponding to the NOEL and LOEL. Maternal blood, urine, and fetal tissue concentrations of EGEE and 2-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 capabilities of the rodent EGEEA and EGEE models to predict the kinetics in humans. The models for estimating circulating blood concentrations of 2-EAA were considered valid based on the ability of the model to accurately predict 2-EAA concentrations in rat blood, urine, and fetal tissue. The human inhaled concentration equivalent to the rat NOEL for EGEEA (50 ppm) was predicted to be 25 ppm using the maternal blood average daily area under the curve (AUC) and 40 ppm using the maximum concentration achieved in maternal blood (C_{max}). The human inhaled concentration equivalent to the rat LOEL for EGEEA (100 ppm) was determined to be 55 ppm using the maternal blood average daily AUC and 80 ppm using the maternal blood C_{max}.**

Key Words: **ethylene glycol monoethyl ether acetate (EGEEA); ethylene glycol monoethyl ether (EGEE); ethoxyacetic acid (EAA); physiologically based pharmacokinetic (PBPK) model.**

Ethylene glycol monoethyl ether (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 and 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).

The current permissible exposure limits (PELs) for occupational exposure, established by the OSHA in 1977, are 200 ppm EGEE and 100 ppm EGEEA, 8-h time-weighted average (TWA_8) (NIOSH, 1997) with skin notations. These standards were established on the basis of blood, kidney, liver, and central nervous system toxicity. OSHA has proposed PELs of 0.5 ppm (TWA_8) for EGEE and EGEEA based on reproductive and developmental toxicity (OSHA, 1993). The threshold limit value (TLV) established for EGEE by the American Conference of Governmental Industrial Hygienists (ACGIH) is 5 ppm (TWA_8) (ACGIH, 1999) and was based on "analogy" to ethylene glycol monomethyl ether (EGME), which also has a TLV of 5 ppm, and evidence that EGEE is less potent in animals than EGME. The TLV for EGEEA is also 5 ppm, based on testicular toxicity to rats and analogy to the EGEE TLV (ACGIH, 1991).

EGEE and EGEEA are known to be developmentally toxic in rodents (Hardin *et al.,* 1982; Andrew and Hardin, 1984; Doe, 1984; Nelson *et al.,* 1984; Kalf *et al.,* 1987; Union Carbide, 1984; Paustenbach, 1988), produce testicular toxicity in male rodents (Cheever *et al.,* 1984; Foster *et al.,* 1984; Kalf *et al.,*

[1] Current address: The Sapphire Group, Twinsburg, Ohio.
[2] Current affiliation: Exponent, Inc., Boulder, Colorado.

0041-008X/00 $35.00

1987; Nagano *et al.,* 1979), and also produce hematological effects (Barbee *et al.,* 1984; Nagano *et al.,* 1979; Union Carbide, 1984). The acid metabolite, 2-ethoxyacetic acid (2-EAA), resulting from metabolism of both EGEE and EGEEA, has been identified as the putative toxicant (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 evaluation of an internal dose of 2-EAA, using measures such as maximum concentration in the blood (C_{max}) and the integrated area under the blood concentration vs time curve (AUC). These internal dose measures have been used for assessing the dose response of EGME and its metabolite, 2-methoxyacetic acid (MAA) (Gargas *et al.,* 2000; Welsch *et al.,* 1995; Clarke *et al.,* 1993; Terry *et al.,* 1995).

The distribution, metabolism, and elimination of EGEEA following pulmonary uptake are the same as for EGEE. Once inhaled, EGEEA is rapidly metabolized 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 metabolism is such that very little EGEEA is present in the blood and distributed to the tissues. EGEE is metabolized via alcohol dehydrogenase and aldehyde dehydrogenase to form 2-EAA and via microsomal oxidation to form EG (Fig. 1). 2-EAA is further metabolized to form a glycine conjugate in rodents (*N*-ethoxyacetyl glycine; EAG) (Kennedy *et al.,* 1993), however, the glycine conjugate of 2-EAA has not been reported in humans (Groeseneken *et al.,* 1988). Both EG and 2-EAA undergo further metabolism to form carbon dioxide. The major metabolites excreted in the urine following an exposure to EGEE are 2-EAA, EG, and EAG (Kennedy *et al.,* 1993).

Physiologically based pharmacokinetic (PBPK) models describing the kinetics of several glycol ethers in rodents and humans are available. Models for EGME have been described for mice (Clarke *et al.,* 1993; Terry *et al.,* 1995; Welsch *et al.,* 1995), rats (Hays *et al.,* 2000; Gargas *et al.,* 2000), and humans (Welsch *et al.,* 1995; Gargas *et al.,* 2000). Welsch *et al.* (1995) and Gargas *et al.* (2000) have used these models to predict acid metabolite concentrations in humans for comparison to the blood concentrations in rats and mice that have produced various developmental effects. No such models currently exist for EGEE or EGEEA. Biologically based risk assessments for these compounds should account for the production, distribution, and elimination of 2-EAA, all processes well suited for evaluation and prediction by pharmacokinetic modeling.

The objectives of the work described here were to conduct a pharmacokinetic study in the rat that provides data sufficient to develop PBPK models for EGEEA and EGEE for pregnant rats and humans and to use those models to estimate human exposures that were equivalent, based on internal 2-EAA dosimetry, to rat exposures resulting in a no observed effect level (NOEL) and a lowest observed effect level (LOEL) for developmental effects in rats. These models are intended to be used in further evaluations to determine safe exposure concentrations in occupational and environmental settings.

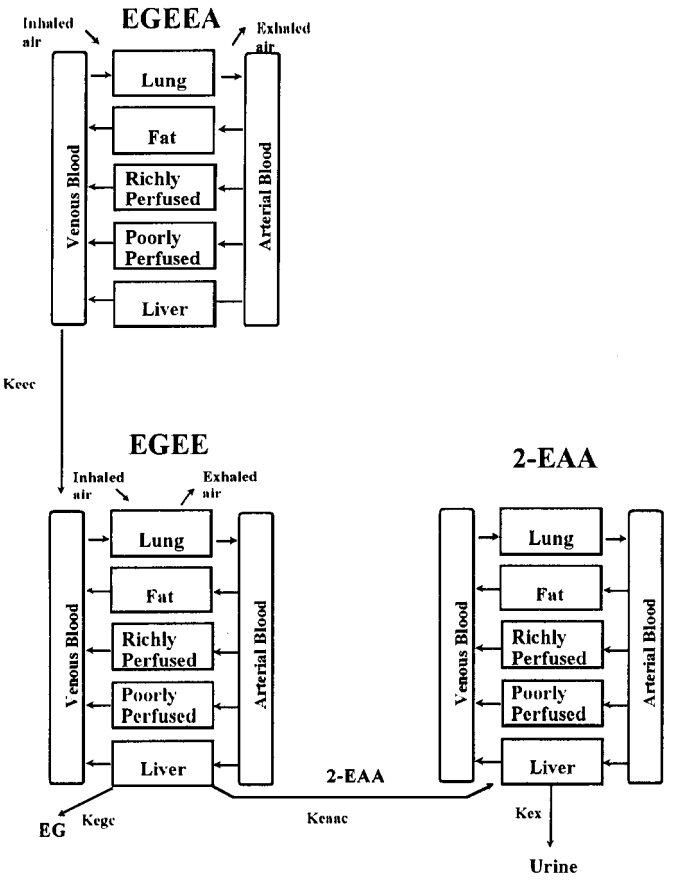

FIG. 1. Schematic of the physiologically based pharmacokinetic description for ethylene glycol monoethyl ether acetate (EGEEA), ethylene glycol monoethyl ether (EGEE), and 2-ethoxy acetic acid (EAA) disposition in pregnant rats and humans. EGEEA is metabolized to EGEE in the blood and EGEE is metabolized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

METHODS

PBPK Models

The PBPK models developed for EGME kinetics in rats and humans (Hays *et al.,* 2000; Gargas *et al.,* 2000) were used as the basis for the EGEEA/EGEE/2-EAA models used in this article. Briefly, a similar model structure was used and contained three submodels (one each for EGEEA, EGEE, and 2-EAA), each with discrete compartments for the lung, liver, fat, and richly and slowly perfused tissues (Fig. 1). All physiological and anatomical parameters, including those that change due to pregnancy, were as described by Hays *et al.* (2000) and Gargas *et al.* (2000). For simplifying purposes, the growing fetus(es) were grouped into the richly perfused tissue compartment based on the observation that rat maternal blood and rat fetal tissue concentrations of 2-MAA were nearly identical (or proportional) following iv and oral exposures to EGME (Clarke *et al.,* 1993; Welsch *et al.,* 1995). 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 fetal disposition of EGEE and 2-EAA are expected to be similar to the disposition of EGME and 2-MAA.

The rat and human tissue:blood partition coefficients for EGEE and 2-EAA were assumed to be the same as for EGME and 2-MAA and were taken directly from Hays *et al.* (2000) (see Table 1). The human blood:air partition coefficients for EGEE were from Johanson and Dynesius (1988). Johanson and Dynesius (1988) were not able to measure a human 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 (35,869 and 23,069, respectively) were very similar to the human blood:air for EGME and EGEE (32,836 and 22,093, respectively), we assumed the saline:air value for EGEEA (Johanson and Dynesius, 1988) was a suitable surrogate for a human blood:air partition coefficient.

The clearance (hydrolysis) of EGEEA in blood to EGEE by blood esterases was initially assumed to be arbitrarily large. However, the rate of decline of the maternal rat blood EGEE concentrations after cessation of EGEEA exposure (collected as described below) was found to be sensitive to this parameter. Thus the clearance of EGEEA via hydrolysis in the rat was identified by fitting the postexposure rat blood EGEE data. For the human, this rate was scaled by body weight to the 0.74 power ($BW^{0.74}$) as is customary when extrapolating clearance across species (Dedrick, 1973; Krishnan and Andersen, 1991).

The first-order rate constants describing the metabolism of EGEE to 2-EAA were derived from Green *et al.* (1996) and adjusted for differences between rate constants in hepatocyte suspensions (Green *et al.*, 1996) and *in vivo* (Corley *et al.*, 1994) for conversion of ethylene glycol monobutyl ether (EGBE) to 2-butoxyacetic acid (see Table 2). The rate of EG production from EGEE was estimated at 30% and 54% of the rates of 2-EAA production in rats and humans, respectively, based on ratios found in rat and human hepatocyte incubations (Green *et al.*, 1996).

The blood concentration time-course data for EGEE and 2-EAA in rats collected during and after inhalation exposure (as described below) were used to estimate the rate of elimination of 2-EAA in the rat urine (K_{ex}; h^{-1}) and as a means of model verification. The rate of urinary elimination of 2-EAA in humans was estimated by comparing model predictions to the data from Groeseneken *et al.* (1986b).

The fractional respiratory bioavailability (*K*alv) was applied to the alveolar ventilation rate, and not pulmonary, as a result of the "wash in–wash out" effect observed with many water-soluble organic vapors. It was not a correction for dead space or a conversion between pulmonary and alveolar ventilation. For highly water-soluble materials, the relative respiratory uptake tends to be less than what would be attributable to alveolar ventilation. It has been suggested that, during inhalation, highly water-soluble solvent vapors are partially adsorbed by mucous membranes and the epithelial lining in the upper and lower respiratory tract, trachea, and bronchiolar region, effectively decreasing the concentration of vapor reaching the alveolar region. Upon exhalation, the solvent vapor diffuses back into the exhaled air, thereby reducing the net systemic bioavailability (Johanson, 1991; Medinsky *et al.*, 1993).

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,b, 1987a,b). 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 4 h with a 50-min on, 10-min off exposure regimen for each h. 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 h postexposure and 2-EAA concentrations were determined following EGEE (Groeseneken *et al.*, 1986b) or EGEEA (Groeseneken *et al.*, 1987a) exposures. Experiment-specific values for body weight, pulmonary ventilation rate, and body fat percentage were used, when available.

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

Rat Data

Experimental design. We evaluated the pharmacokinetics of EGEEA in pregnant Sprague–Dawley rats following whole body vapor inhalation exposures. The rats were exposed to two concentrations of EGEEA that corresponded to the NOEL, 50 ppm (Doe, 1984; OSHA, 1993), and LOEL, 100 ppm (OSHA, 1993; Union Carbide, 1984), for developmental toxicity in rats. The animals were exposed for 6 h/day for 5 consecutive days on gestation days (GD) 11–15. Subgroups of four rats/time period were euthanized during and after the final exposure for the analysis of EGEEA and 2-EAA in blood, urine, and fetal samples (each litter pooled and homogenized). Exposures were conducted at Battelle Pacific Northwest Laboratories (Richland, WA).

Due to the rapid hydrolysis of the acetate moieties of glycol ethers (Stott and McKenna, 1985; Groeseneken *et al.*, 1987a,b), analytical methods were optimized for the quantitation of the parent glycol ether, EGEE, and its metabolite, 2-EAA, 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 2-EAA.

Chemicals. EGEEA was used as supplied by Union Carbide (Charleston, WV). The purity of each test material reported by the supplier was ≥99.8% with a water content of ≤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 5 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 h 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 were 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 ≤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 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

TABLE 1
Parameters for EGEEA PBPK Models

Parameter	Value: Rat	Value: Human	Parameter estimation
Weights			
Body weight - 50-ppm-exposed group	bw = 0.3851 * T + 124.77		Measured[a]
Body weight - 100-ppm-exposed group	bw = 0.2017 * T + 193.76		Measured[a]
Body weight (start and end of pregnancy)		58, 62 kg	Fixed[b]
Percentage of body weight	**GD 13**	**Day 0**	
Liver	4.0	2.4	Measured
Fat	10.1	27.6	Measured[c]
Slowly perfused	65.0	48.7	Measured
Richly perfused	6.1	3.7	Measured[d]
Blood	5.9	5.9	Fixed[e]
Flows			
Cardiac output (liters/h/kg$^{0.74}$)	14.0	19.2	Fixed
Alveolar ventilation (liters/h/kg$^{0.74}$)	14.0	15.3	Fixed
Percentage of cardiac output (%)	**GD 13**	**Day 0**	
Liver	25.0	24.0	Fixed
Fat	14.2	9.5	Fixed[f]
Slowly perfused	15.0	19.0	Fixed
Richly perfused	45.8	47.5	Fixed[g]

Partition coefficients	EGEEA	EGEE	EAA	Parameter estimation
Blood:air	3822	22093	NA	Measured[h]
Liver:blood	1	1	1.1	Fixed[i]
Fat:blood	1.3	0.04	0.32	Measured[j]
Slowly perfused:blood	0.94	0.94	0.5	Fixed[i]
Richly perfused:blood	1.1	1.1	1.05	Fixed[i]

Metabolic constants	Rat	Human	Parameter estimation
K_{eec} (L blood/h) EGEEA → EGEE	2.3	2.3	Fitted[k]
K_{eaac} (L/h/kg liver) EGEE → EAA	223	76.6	Fixed[l]
K_{egc} (L/h/kg liver) EGEE → EG	66.9	41.4	Fixed[m]
Urinary excretion			
K_{ex} (L/h)	0.015	0.4	Fitted[n]
Alveolar absorption factor			
K_{alv} (unitless)	0.65	0.65	Fixed[o]

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

[b] 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).

[c] Includes growing mammary tissue (Knight *et al.*, 1984; Luecke *et al.*, 1994).

[d] Includes growing placenta and fetus(es) (Fisher *et al.*, 1989; Hays *et al.*, 2000; Luecke *et al.*, 1994).

[e] From Andersen *et al.* (1991).

[f] Includes increasing flow to mammary tissue (Fisher *et al.*, 1989; Metcalfe *et al.*, 1988).

[g] Includes flow to placenta and fetus, calculated as 100% flows to other tissues.

[h] Johanson and Dynesius (1988). Value for EGEEA is for saline:air. Value for EGEE is for blood:air (see text for explanation).

[i] The measured partition coefficients for EGME were used for EGEEA and EGEE and the partition coefficients for MAA were used for EAA (Hays *et al.*, 2000).

[j] Johanson and Dynesius (1988). Value for EGEEA is value reported for oil:water and value for EGEE was value reported for oil:blood (see text for explanation).

[k] Optimized in rat by fit of postexposure blood EGEE concentration.

[l] Green *et al.* (1996), adjusted for *in vitro–in vivo* differences in reaction rates (see text for explanation).

[m] Tyson *et al.* (1989), adjusted for *in vitro–in vivo* differences in reaction rates (see text for explanation).

[n] Optimized by fitting model output to blood concentrations or urinary output of EAA (see text for discussion).

[o] Groeseneken *et al.* (1987b).

TABLE 2
First Order Reaction Rate Constants for Conversion of Glycol Ether to Alkoxyacetic Acid

Parent compound	Species	Experimental system	Rate constant (L/h/kg of liver)	*In vivo/in vitro* ratio
Ethylene glycol monobutyl ether (EGBE)	Rat	*In vitro*	113[a]	7.57
	Rat	*In vivo* (surrogate)	856[b]	
	Human	*In vitro*	12.05[a]	10.2
	Human	*In vivo* (surrogate)	124.1[b]	
EGEE	Rat	*In vitro*	29.5[a]	
	Rat	*In vivo* (predicted)	29.5 * 7.57 = 223[c]	
	Human	*In vitro*	7.5[a]	
	Human	*In vivo* (predicted)	7.5 * 10.2 = 76.6[c]	

[a] From Green *et al.* (1996), converted from cell number to liver weight (128×10^6 cells/g of liver) (Seglen, 1976).
[b] From Corley *et al.* (1994), isolated perfused rat liver studies, scaled for a 0.23-kg rat or 70-kg human and used as a surrogate for *in vivo* metabolism.
[c] *In vitro* rate for EGEE multiplied by *in vivo/in vitro* ratio calculated for EGBE.

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 2-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 0.9 M sulfuric acid, addition of 0.25 g sodium sulfate (to improve extraction efficiencies), and extraction with 0.5 ml ethyl acetate. Separation and quantitation was achieved using a 30 m $\times$ 0.32 mm id $\times$ 0.5-μm film thickness Stabilwax DA capillary column (Restek) and a Hewlett Packard 6890 GC/FID. Splitless injections of $\sim$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 and then was increased at 20°C/min to 240°C and held for 1 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 2-EAA, 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 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. Methoxyethanol and methoxyacetic acid were used as internal standards for EGEE and 2-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; the detection limit for EGEEA was 0.018 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 h during the exposure, immediately post-exposure, and at 0.5, 1, 2, 4, 8, 18, 30, and 42 h postexposure. Blood samples were collected from control animals, spiked with known quantities of EGEE and 2-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 2-EAA 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°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 h postexposure were analyzed for EGEE and 2-EAA as described above.

Estimating Equivalent Internal Doses

The PBPK model for the rat was used to predict the expected peak (C_{max}) and average area under the blood concentration curve (AUC) for 2-EAA in the rat under the conditions of the critical toxicology study (Doe, 1984). That is, the rats were exposed 6 h/day on GD 6–15. C_{max} and the average daily blood AUC of 2-EAA during GD 13–15 were computed. The choice of GD 13–15 was based on analogy to 2-methoxyethanol; the occurrence of malformations and number of live embryos/litter were maximized in rats dosed intravenously with 500 mg 2-methoxyethanol/kg body weight on GD 13–15 (Sleet *et al.*, 1996). These same values for C_{max} and average daily AUC in the rat were used as the target values for predictions using the human pregnancy model. Various inhaled concentrations of EGEEA for 8-h periods were used as inputs to the human model until C_{max} and average daily AUC values for 2-EAA in blood were determined to be equal to those determined for rats. The resulting inhaled EGEEA concentrations for humans were then considered to be equivalent to the NOEL and LOEL in rats for developmental effects, based on the inference that these are the most appropriate predictors of susceptibility, dose, and toxicity across species.

RESULTS

Animal Experiments

The average concentrations of EGEEA in the chambers, over the 30 h of exposure (over 5 days) were 47.5 and 108.2 ppm for the target concentrations of 50 and 100 ppm, respectively, as determined by GC-FID. The average daily chamber

concentrations were within ±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. This finding was consistent with previous studies (Hanley *et al.*, 1984).

Model Predictions of Rat Data

Since the half-life of 2-EAA in urine is on the order of 9–24 h in rats (Cheever *et al.*, 1984; Medinsky *et al.*, 1990), exposures to EGEEA 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 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 5 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 2-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 (MGA Software, 1995).

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 exposure concentrations, steady-state blood concentrations appear to have been reached for both exposure concentrations. Peak EGEE blood concentrations of 0.7 and 2.3 mg/L (Fig. 2A) were measured at the end of the final exposures on GD 15 for the 50- and 100-ppm exposures, respectively. Model predictions of the rat data compared very well to measured EGEE maternal blood concentrations at both 50 and 100 ppm (Fig. 2A). For the major metabolite, 2-EAA, peak blood concentrations of 24.7 and 73.5 mg/L (Fig. 2B) were measured at the end of the 50- and 100-ppm exposures, respectively. The predicted maternal rat blood EGEE concentrations in the postexposure period, but not during exposure, were found to be sensitive to clearance of EGEEA due to hydrolysis. This clearance was optimized based on the log likelihood function (Steiner *et al.*, 1990) and a very good fit was achieved, especially for the 50-ppm EGEEA exposure. The PBPK model provided reasonable predictions of the 2-EAA concentration time-courses for both exposure concentrations in rats (Fig. 2B).

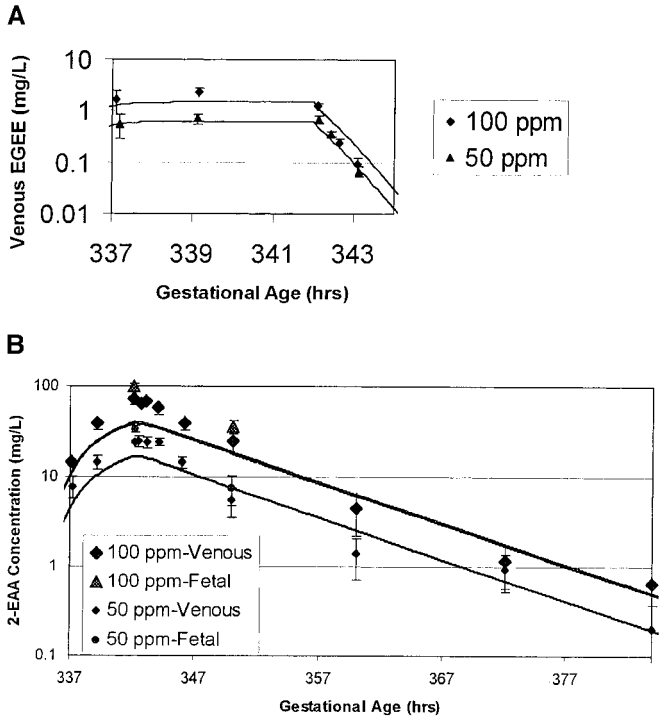

FIG. 2. Concentrations in maternal venous blood and fetal tissue during and following GD 15 ($t = 336$ h) for pregnant rats exposed to 100 ppm (upper lines) or 50 ppm (lower lines) EGEEA on GD 11–15. Values are the mean ± SD of up to four rats (or the fetuses from four rats)/sample time. The smooth curves are those predicted by the PBPK model. (A) Concentrations of EGEE. (B) Concentrations of 2-EAA.

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 h postexposure. 2-EAA concentrations in rat fetuses averaged 40% higher than the corresponding rat maternal blood concentrations at 0 and 8 h postexposure (Fig. 2B). As was found with EGME and 2-MAA, this proportionality did not appear to be concentration or time dependent (Gargas *et al.*, 2000).

The excretion rate of 2-EAA 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-EAA concentrations was produced. This value was confirmed as optimal using a well-accepted optimization criterion, maximization of the log likelihood function (Steiner *et al.*, 1990).

The model predicts that 2.13 and 5.04 mg 2-EAA will be excreted in the urine after exposure to 50 and 100 ppm EGEEA, respectively. Postexposure urinary excretion of 2-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 2-EAA-

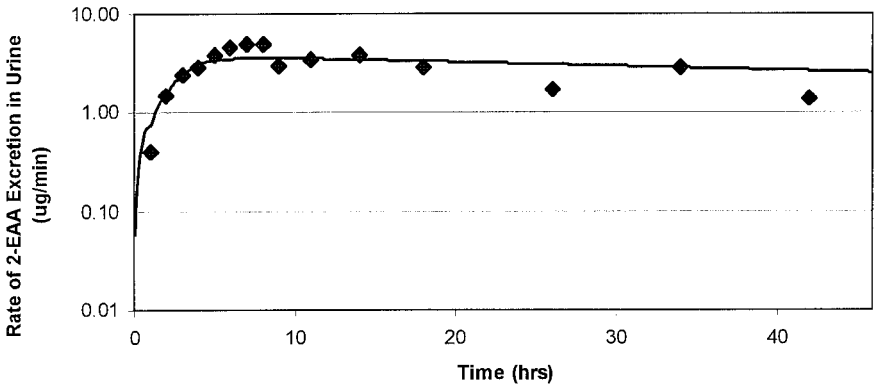

FIG. 3. The rate of urinary excretion of 2-EAA from human volunteers ($n = 5$) exposed to 20 mg EGEE/m^3 (four 50-min exposures with a 10-min break for urine collection at the end of each 50-min exposure) (Groeseneken *et al.,* 1988). The smooth curve was produced by the human PBPK model by adjusting K_{ex} until a reasonable fit was achieved ($K_{ex} = 0.4$ L/h). Average pulmonary ventilation rate (QPC = 33 L/h/kg$^{0.74}$), body weight (70 kg), and fraction of body weight as fat (0.12) reported by Groeseneken *et al.* (1988) were used in model predictions.

derived urinary metabolites, and the analytical method measures only 2-EAA. Experimental work (Cheever *et al.,* 1984; Groeseneken *et al.,* 1988; Sabourin *et al.,* 1992; Kennedy *et al.,* 1993) indicates that 2-EAA-derived urinary metabolites such as glycine conjugates may contribute 14 to 74% more 2-EAA-derived urinary metabolites. The average in studies with male SD rats, the strain used in the study reported here, was 68% (Cheever *et al.,* 1984). It was therefore estimated that measured amounts of urinary 2-EAA should be multiplied by a factor of 1.7 to approximate the total flux through the 2-EAA pathway (amount of unchanged 2-EAA plus glycine conjugates and other metabolites). The resulting estimates of 1.77- and 4.81-mg 2-EAA-derived urinary metabolites were reasonably close to model predictions.

Model Predictions of Human Data

The first-order rate constant (K_{ex}) that describes the urinary elimination of 2-EAA was varied until reasonable fits, as determined by visual inspection, were achieved to the data following EGEE exposures from Groeseneken *et al.* (1986b) (see Fig. 3; only 20 mg/m^3 data shown, 10 and 40 mg/m^3 data were fit equally as well). This value was confirmed as optimal using the log likelihood function (Steiner *et al.,* 1990). This parameter, as well as all others, were held constant (Table 1) for the further model predictions of the urinary excretion of 2-EAA following EGEEA exposures. A reasonable fit (Fig. 4; only 28 mg/m^3 data shown, 14 and 50 mg/m^3 data were fit equally as well) was achieved to the urinary excretion of 2-EAA following EGEEA exposures to male human volunteers (Groeseneken *et al.,* 1987a), indicating that the optimized value for K_{ex} is reasonable.

Model predictions of exhaled breath concentrations of EGEE provided good fits to the data of Groeseneken *et al.* (1986) (Fig. 5; data and fit shown for 20 mg/m^3 exposure only; similar results were obtained for 10 and 40 mg/m^3 exposures). The model did predict a more rapid decline of exhaled breath

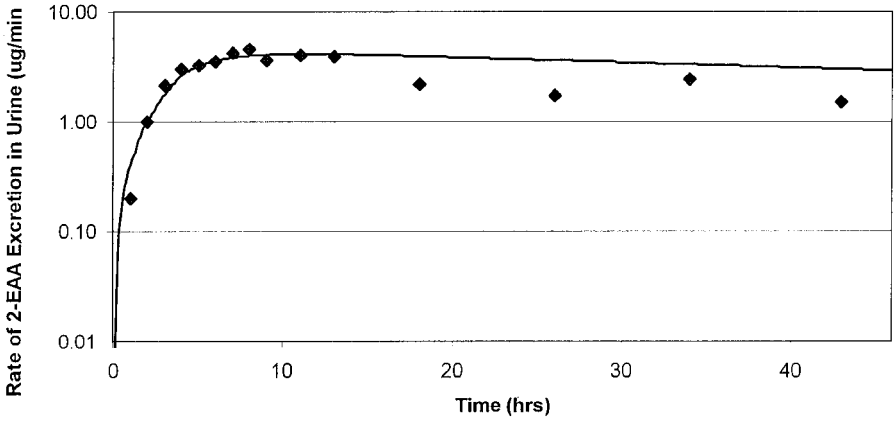

FIG. 4. The rate of urinary excretion of 2-EAA from human volunteers ($n = 5$) exposed to 28 mg EGEEA/m^3 (four 50-min exposures with a 10-min break for urine collection at the end of each 50-min exposure) (Groeseneken *et al.,* 1987a). The smooth curve was produced by the human PBPK model. Average pulmonary ventilation rate (QPC = 36 L/h/kg$^{0.74}$), body weight (68 kg), and fraction of body weight as fat (0.09) reported by Groeseneken *et al.* (1987b) were used in model predictions.

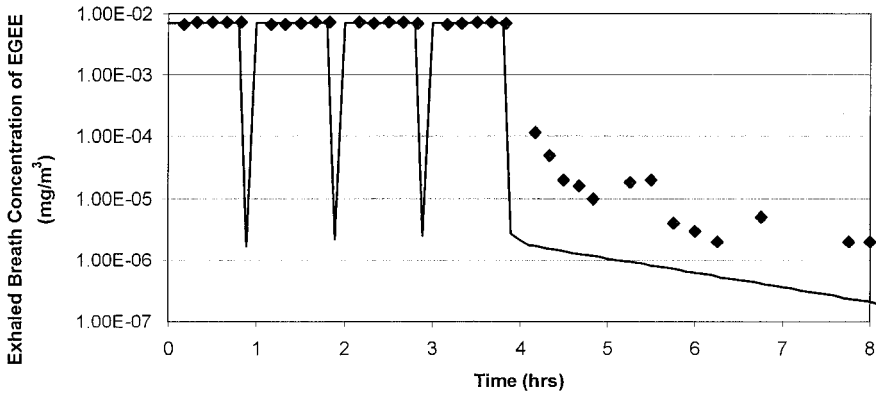

FIG. 5. Exhaled breath concentrations of EGEE from human volunteers ($n = 5$) exposed to 20 mg EGEE/m^3 (four 50-min exposures with a 10-min break for urine collection at the end of each 50-min exposure) (Groeseneken *et al.*, 1988). Average pulmonary ventilation rate (QPC = 33 L/h/kg$^{0.74}$), body weight (70 kg), and fraction of body weight as fat (0.12) reported by Groeseneken *et al.* (1988) were used in model predictions.

concentrations at the end of exposure than was observed. Our model was able to successfully predict the exhaled breath concentration data for EGEEA (Groeseneken *et al.*, 1987b) during exposure and the model somewhat underpredicted the postexposure exhaled breath concentration of EGEEA (Fig. 6; data and fit shown for 28 mg/m^3 exposure only; similar results were obtained for 14 and 50 mg/m^3 exposures). In addition, the model underpredicted EGEE exhaled breath concentrations measured during EGEEA exposures by slightly more than a factor of 10 (data and predictions not shown). Although in absolute terms this is an appreciable difference, from a mass balance standpoint less than 1% of the inhaled EGEEA is eliminated as EGEE in exhaled breath; thus, the lack of fit does not reflect unfavorably on the ability of the model to describe the kinetics in humans.

Equivalent Internal Dosimetry

The PBPK model for the pregnant rat was used to determine venous blood C_{max} and average AUC for 2-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 2-EAA blood and fetus concentrations (Fig. 2B). Maternal blood concentrations of 2-EAA were considered appropriate surrogates for fetal 2-EAA concentrations since the concentrations appear to be proportional, although not identical. The human pregnancy model was used to predict these maternal blood 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 EGEEA. Our results indicate that if a pregnant woman were exposed to 25 ppm EGEEA, this would produce circulating blood concentrations of 2-EAA that would yield a daily blood AUC equal to the daily blood AUC for 2-EAA that occurred when the rat was exposed to 50 ppm (the NOEL for developmental effects). Likewise our results indicate that if a pregnant woman were exposed to 40 ppm EGEEA, then this would result in the same peak blood concentration of 2-EAA as that

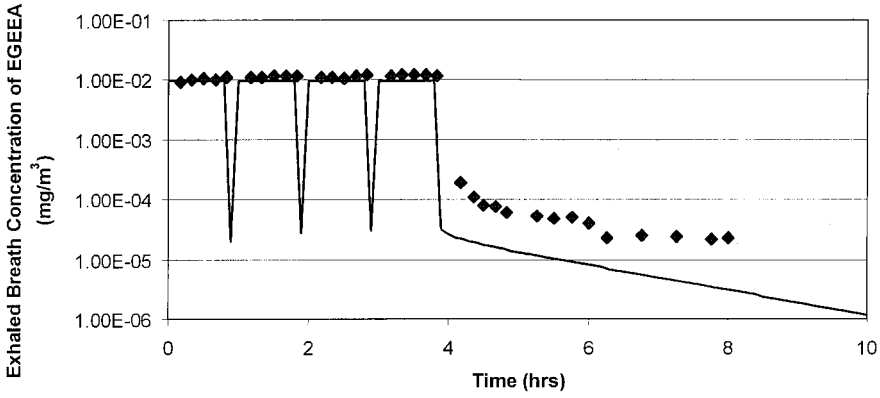

FIG. 6. Exhaled breath concentrations of EGEEA from human volunteers ($n = 5$) exposed to 28 mg EGEEA/m^3 (four 50-min exposures with a 10-min break for urine collection at the end of each 50-min exposure) (Groeseneken *et al.*, 1987b). Average pulmonary ventilation rate (QPC = 36 L/h/kg$^{0.74}$), body weight (68 kg), and fraction of body weight as fat (0.09) reported by Groeseneken *et al.* (1987b) were used in model predictions.

occurring in a rat exposed to 50 ppm. For the rat developmental toxicity LOEL (100 ppm), the human equivalent concentrations are 55 ppm for average daily blood AUC and 80 ppm for peak blood concentration.

DISCUSSION

The model predictions of the rat data for EGEE and 2-EAA in maternal blood and 2-EAA in fetal tissue after exposure to EGEEA were very good for both inhaled concentrations (Fig. 2). This accomplishment is significant because the only adjusted parameters were the urinary excretion rate of 2-EAA and clearance of EGEEA via hydrolysis. 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 basis of the concentration differences seen between maternal blood 2-EAA and fetal homogenate 2-EAA have not yet been elucidated. It is possible that solubility differences play a role, but partition coefficients were not directly measured in this work. It is important to note, however, that the maternal blood and fetal homogenate 2-EAA concentrations were proportional and did not appear to be concentration or time dependent, adding confidence to our use of 2-EAA dose metrics for determining equivalent human exposure concentrations at the rat NOEL and LOEL.

The clearance of EGEEA in rat blood was determined by optimization when it was found that postexposure rat blood EGEE concentrations were quite sensitive to this parameter. EGEEA was not detected in the blood of rats exposed to 100 ppm EGEEA, even though the model predicts it should have been present at concentrations above the limit of detection. It is possible that a portion of the EGEE measured in rat blood may have been produced by hydrolysis of EGEEA during the brief interval between when inhalation ended and the plasma esterases were inactivated in the freezing process thus overestimating the amount present during exposure (2 min would be sufficient to reduce EGEEA concentrations below the limit of detection). While this may decrease confidence in the use of EGEEA component as a "stand alone" model, it does not impact the predictions of 2-EAA kinetics and thus does not effect the utility of the EGEEA/EGEE/2-EAA model for risk assessment.

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 2-EAA after EGEE exposure (Fig. 3) worked very well at predicting 2-EAA excretion after EGEEA exposure (Fig. 4). 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. 5). Predictions of EGEEA in exhaled breath were good during exposure but were somewhat underpredicted in the postexposure period (Fig. 6). There are a number of reasons why the prediction of the concentration of EGEE (and EGEEA, postexposure) in exhaled breath was less than optimal. First, it is difficult to accurately measure such low concentrations of EGEEA and EGEE. Second, the water:air partition coefficient for EGEEA was used in place of a blood:air partition coefficient. Third, the "wash in–wash out" effect may be significant. This phenomenon has been observed by several others (Gerde and Dahl, 1991; Johanson, 1991; Medinsky *et al.*, 1993). They found that, after inhalation exposure to a water-soluble compound like EGEEA, the mucous membranes release (due to diffusion) some of the chemical that has been absorbed by the cells during the exposure period. This produces a "tail" in the exhaled breath concentration vs time curve. It is important to note, however, that data involving the presumed developmentally toxic form of the chemical, 2-EAA, were all well fit by both the rodent and human models.

The Green *et al.* (1996) *in vitro* hepatocyte metabolism study provided a direct comparison of rat and human metabolic rates for both EGBE and EGEE using intact hepatocytes. As a first approximation for estimating *in vivo* rat and human metabolic rate constants for EGEE, we assumed that the ratio (not absolute levels) of *in vivo* to *in vitro* clearances (V_{max}/K_m ratios) for EGBE metabolism to butoxyacetic acid would be equivalent to metabolism of EGEE to ethoxyacetic acid, since they are metabolized via the same enzymes (alcohol and aldehyde dehydrogenases). The alternative, simply scaling up directly from the *in vitro* to *in vivo* based on hepatocyte numbers, resulted in overprediction of blood EGEE concentrations (data not shown). This approach was considered reasonable since no *in vivo* parameters were available and it minimized the numbers of parameters that required adjusting to fit *in vivo* data. *In vivo* human data for estimating model parameters or for model validation are usually quite scarce, and thus the development of *in vitro* techniques that support the modeling of *in vivo* observations in humans will become increasingly important.

The use of internal dose 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 dose of EGEEA metabolites lends a degree of confidence to use model-estimated internal doses 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 developmental effects of EGEEA in rodents, it would be prudent at this time to consider 25 and 55 ppm as being the human-equivalent NOEL and LOEL concentrations for EGEEA.

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.

Andersen, M. E., Clewell III, H. J., Gargas, M. L., MacNaughton, M. G., Reitz, R. H., Nolan, R. J., and McKenna, M. J. (1991). Physiologically based pharmacokinetic modeling with dichloromethane, its metabolite, carbon monoxide, and blood carboxyhemoglobin in rats and humans. *Toxicol. Appl. Pharmacol.* **108,** 14–27.

Andrew, F. D., and Hardin, B. D. (1984). Developmental effects after inhalation exposure of gravid rabbits and rats to ethylene glycol monoethyl ether. *Environ. Health Perspect.* **57,** 13–23.

Barbee, S. J., Terrill, J. B., DeSousa, D. J., and Conaway, C. C. (1984). Subchronic inhalation toxicology of ethylene glycol monoethyl ether in the rat and rabbit. *Environ. Health Perspect.* **57,** 157–163.

Cheever, K. L., Plotnick, H. B., Richards, D. E., and Weigel, W. W. (1984). Metabolism and excretion of 2-ethoxyethanol in the adult male rat. *Environ. Health Perspect.* **57,** 241–248.

Clarke, D. O., Elswick, B. A., Welsch, F., and Conolly, R. B. (1993). Pharmacokinetics of 2-methoxyethanol and 2-methoxyacetic acid in the pregnant mouse: A physiologically based mathematical model. *Toxicol. Appl. Pharmacol.* **121,** 239–252.

Corley, R. A., Bormett, G. A., and Ghanayem, B. I. (1994). Physiologically based pharmacokinetics of 2-butoxyethanol and its major metabolite, 2-butoxyacetic acid, in rats and humans. *Toxicol. Appl. Pharmacol.* **129,** 61–79.

Dedrick, R. L. (1973). Animal scale-up. *J. Pharmacokin. Biopharm.* **1**(5), 435–461.

Doe, J. E. (1984). Ethylene glycol monoethyl ether and ethylene glycol monoethyl ether acetate teratology studies. *Environ. Health Perspect.* **57,** 33–41.

Fisher, J. W., Whittaker, T. A., Taylor, D. H., Clewell, H. J., III, and Andersen, M. E. (1989). Physiologically based pharmacokinetic modeling of the pregnant rat: A multiroute exposure model for trichloroethylene and its metabolite, trichloroacetic acid. *Toxicol. Appl. Pharmacol.* **99,** 395–414.

Foster, P. M., Creasy, D. M., Foster, J. R., and Gray, T. J. (1984). Testicular toxicity produced by ethylene glycol monomethyl and monoethyl ethers in the rat. *Environ. Health Perspect.* **57,** 207–217.

Gargas, M. L., Tyler, T. R., Sweeney, L. M., Corley, R. A., Weitz, K. K., Mast, T. J., Paustenbach, D. J., and Hays, S. M. (2000). 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. *Toxicol. Appl. Pharmacol.* **165,** 53–62.

Gerde, P., and Dahl, A. (1991). A model for the uptake of inhaled vapors in the nose of the dog during cyclic breathing. *Toxicol. Appl. Pharmacol.* **109,** 276–288.

Green, C., Gordon, G., Cohen, P., Nolen, H., Peters, J., and Tyson, C. (1996). In vitro metabolism of glycol ethers by human and rat hepatocytes. *Occup. Hyg.* **2,** 67–75.

Groeseneken, D., Veulemans, H., and Masschelein, R. (1986a). Respiratory uptake and elimination of ethylene glycol monoethyl ether after experimental human exposure. *Br. J. Ind. Med.* **43,** 544–549.

Groeseneken, D., Veulemans, H., and Masschelein, R. (1986b). Urinary excretion of ethoxyacetic acid after experimental human exposure to ethylene glycol monoethyl ether. *Br. J. Ind. Med.* **43,** 615–619.

Groeseneken, D., Veulemans, H., Masschelein, R., and Van Vlem, E. (1987a). Ethoxyacetic acid: A metabolite of ethylene glycol monoethyl ether acetate in man. *Br. J. Ind. Med.* **44,** 488–493.

Groeseneken, D., Veulemans, H., Masschelein, R., and Van Vlem, E. (1987b). Pulmonary absorption and elimination of ethylene glycol monoethyl ether acetate in man. *Br. J. Ind. Med.* **44,** 309–316.

Groeseneken, D., Veulemans, H., Masschelein, R., and Van Vlem, E. (1988). Comparative urinary excretion of ethoxyacetic acid in man and rat after single low doses of ethylene glycol monoethyl ether. *Toxicol. Lett.* **41,** 57–68.

Guest, D., Hamilton, M. L., Deisinger, P. J., and DiVincenzo, G. D. (1984). Pulmonary and percutaneous absorption of 2-propoxyethyl acetate and 2-ethoxyethyl acetate in beagle dogs. *Environ. Health Perspect.* **57,** 177–183.

Hanley, T. R., Jr., Yano, B. L., Nitschke, K. D., and John, J. A. (1984). Comparison of the teratogenic potential of inhaled ethylene glycol monomethyl ether in rats, mice, and rabbits. *Toxicol. Appl. Pharmacol.* **75,** 409–422.

Hardin, B. D., Niemeier, R. W., Smith, R. J., Kuczuk, M. H., Mathinos, P. R., and Weaver, T. F. (1982). Teratogenicity of 2-ethoxyethanol by dermal application. *Drug Chem. Toxicol.* **5,** 277–294.

Hays, S. M., Elswick, B. A., Blumenthal, G. M., Welsch, F., Conolly, R. B., and Gargas, M. L. (2000). Development of a physiologically based pharmacokinetic model of 2-methoxyethanol and 2-methoxyacetic acid disposition in pregnant rats. *Toxicol. Appl. Pharmacol.* **163,** 67–74.

Hays, S. M., Long, T. M., Kirman, C. R., and Gargas, M. L. (1998). Potential uses of PBPK modeling to improve the regulation of exposure to toxic compounds. *Inside EPA's Risk Policy Report* **5**(12), 37–41.

International Commission on Radiological Protection (ICRP) (1975). *Report of the Task Group on Reference Man.* ICRP No. 23. Pergamon Press, Elmsford, New York.

Johanson, G. (1991). Modelling of respiratory exchange of polar solvents. *Am. Occup. Hyg.* **35,** 323–339.

Johanson, G., and Dynesius, B. (1988). Liquid/air partition coefficients of six commonly used glycol ethers. *Br. J. Ind. Med.* **45,** 561–564.

Kalf, G., Post, G., and Snyder, R. (1987). Solvent toxicology: Recent advances in the toxicology of benzene, the glycol ethers, and carbon tetrachloride. *Annu. Rev. Pharmacol. Toxicol.* **27,** 399–427.

Kennedy, C. H., Bechtold, W. E., Chang, I. Y., and Henderson, R. F. (1993). Effect of dose on the disposition of 2-ethoxyethanol after inhalation by F344/N rats. *Fundam. Appl. Toxicol.* **21,** 486–491.

Kezic, S., Mahieu, K., Monster, A. C., and de Wolff, F. A. (1997). Dermal absorption of vaporous and liquid 2-methoxyethanol and 2-ethoxyethanol in volunteers. *Occup. Environ. Med.* **54,** 38–43.

Knight, C. H., Docherty, A. H., and Peaker, M. (1984). Milk yield in rats in relation to activity and size of the mammary secretory cell population. *J. Dairy Res.* **51,** 29–35.

Krishnan, K., and Andersen, M. E. (1991). Interspecies scaling in pharmacokinetics. In *New Trends in Pharmacokinetics* (A. Rescigno and A. K. Thakur, Eds.). Plenum Press, New York.

Luecke, R. H., Wosilait, W. D., Pearce, B. A., and Young, J. F. (1994). A physiologically based pharmacokinetic computer model for human pregnancy. *Teratology* **49,** 90–103.

Medinsky, M., Kimbell, J., Morris, J., Gerde, P., and Overton, J. (1993). Symposium overview: Advances in biologically based models for respiratory tract uptake of inhaled volatiles. *Fundam. Appl. Toxicol.* **20,** 265–272.

Medinsky, M. A., Singh, G., Bechtold, W. E., Bond, J. A., Sabourin, P. J., Birnbaum, L. S., and Henderson, R. F. (1990). Disposition of three glycol ethers administered in drinking water to male F344/N rats. *Toxicol. Appl. Pharmacol.* **102**(3), 443–455.

Metcalfe, J., Stock, M. K., and Barron, D. H. (1988). Maternal physiology during gestation. In *Physiology of Reproduction* (E. Knobil and J. D. Neill, Eds.), pp. 2145–2176. Raven Press, New York.

Nagano, K. E., Nakayama, E., Koyano, M., Oobayashi, H., Adachi, H., and Yamada, T. (1979). Testicular atrophy of mice induced by ethylene glycol monoalkyl ethers. *Jpn. J. Ind. Health* **21,** 29–35.

Nelson, B. K., Brightwell, W. S., Setzer, J. V., and O.D. T.L. (1984). Reproductive toxicity of the industrial solvent 2-ethoxyethanol in rats and interactive effects of ethanol. *Environ. Health Perspect.* **57,** 255–259.

NIOSH (1997). NIOSH pocket guide to chemical hazards. U.S. Department of Health and Human Services, Washington, D.C.

OSHA (1993). Occupational exposure to 2-methoxyethanol, 2-ethoxyethanol and their acetates (glycol ethers); proposed rule. *29 CFR Part 1910* **58**(54), 15526–15632.

Paustenbach, D. J. (1988). Assessment of the development risks resulting from occupational exposure to select glycol ethers within the semi-conductor industry. *J. Toxicol. Env. Health* **23,** 53–96.

Sabourin, P. J., Medinsky, M. A., Thurmond, F., Birnbaum, L. S., and Henderson, R. F. (1992). Effect of dose on the disposition of methoxyethanol, ethoxyethanol, and butoxyethanol administered dermally to male F344/N rats. *Fundam. Appl. Toxicol.* **19,** 124–132. [published erratum appears in *Fundam. Appl. Toxicol.* 1993; **20,** 508–510]

Seglen, P. O. (1976). Preparation of isolated rat liver cells. *Methods Cell Biol.* **13,** 29–84.

Sleet, R. B., Welsch, F., Myers, C. B., and Marr, M. C. (1996). Developmental phase specificity and dose–response effects of 2-methoxyethanol in rats. *Fundam. Appl. Toxicol.* **29,** 131–139.

Steiner, E. C., Rey, T. D., and McCroskey, P. S. (1990). Reference Guide: SimuSolv Modeling and Simulation Software. Dow Chemical Company, Midland, MI.

Stott, W. T., and McKenna, M. J. (1985). Hydrolysis of several glycol ether acetates and acrylate esters by nasal mucosal carboxylesterase in vitro. *Fundam. Appl. Toxicol.* **5**(2), 399–404.

Terry, K., Elswick, B., Welsch, F., and Conolly, R. (1995). Development of a physiologically based pharmacokinetic model describing 2-methoxyacetic acid disposition in the pregnant mouse. *Toxicol. Appl. Pharmacol.* **132,** 103–114.

Tyson, C., Green, C., and Peters, J. (1989). *Method Development To Assess Human Metabolism of Chemical Xenobiotics.* SRI International, Menlo Park, CA.

Union Carbide. (1984). A Teratologic Evaluation of CELLOSOLVE Acetate in Fischer 344 Rats and New Zealand White Rabbits Following Inhalation Exposure. Bushy Run Research Center, Export, PA., Project Report 47-116.

Welsch, F., Blumenthal, G. M., and Conolly, R. B. (1995). Physiologically based pharmacokinetic models applicable to organogenesis: Extrapolation between species and potential use in prenatal toxicity risk assessments. *Toxicol. Lett.* **82-83,** 539–547.