TOXICOLOGICAL SCIENCES **85**, 476–490 (2005)
doi:10.1093/toxsci/kfi119
Advance Access publication February 16, 2005

Development of a Physiologically Based Pharmacokinetic Model for Ethylene Glycol and Its Metabolite, Glycolic Acid, in Rats and Humans

R. A. Corley,*[,1] M. J. Bartels,† E. W. Carney,† K. K. Weitz,* J. J. Soelberg,* R. A. Gies,* and K. D. Thrall*

Battelle Pacific Northwest Division, Richland, Washington 99352; †The Dow Chemical Company, Midland, Michigan 48674

Received October 26, 2004; accepted February 12, 2005

An extensive database on the toxicity and modes of action of ethylene glycol (EG) has been developed over the past several decades. Although renal toxicity has long been recognized as a potential outcome, in recent years developmental toxicity, an effect observed only in rats and mice, has become the subject of extensive research and regulatory reviews to establish guidelines for human exposures. The developmental toxicity of EG has been attributed to the intermediate metabolite, glycolic acid (GA), which can become a major metabolite when EG is administered to rats and mice at high doses and dose rates. Therefore, a physiologically based pharmacokinetic (PBPK) model was developed to integrate the extensive mode of action and pharmacokinetic data on EG and GA for use in developmental risk assessments. The resulting PBPK model includes inhalation, oral, dermal, intravenous, and subcutaneous routes of administration. Metabolism of EG and GA were described in the liver with elimination via the kidneys. Metabolic rate constants and partition coefficients for EG and GA were estimated from *in vitro* studies. Other biochemical constants were optimized from appropriate *in vivo* pharmacokinetic studies. Several controlled rat and human metabolism studies were used to validate the resulting PBPK model. When internal dose surrogates were compared in rats and humans over a broad range of exposures, it was concluded that humans are unlikely to achieve blood levels of GA that have been associated with developmental toxicity in rats following occupational or environmental exposures.

Key Words: **ethylene glycol; glycolic acid; PBPK modeling.**

INTRODUCTION

Ethylene glycol (EG) is a high production volume (HPV) chemical used primarily in the synthesis of polyethylene terephthalate (PET) resins, unsaturated polyester resins, polyester fibers, and films (SRI, 2003). Perhaps the better-known uses and potential for occupational or consumer exposures to EG are as a constituent in antifreeze, deicing fluids, heat transfer fluids, industrial coolants, and hydraulic fluids, which accounts for approximately one third of the U.S. production (SRI, 2003).

Given the many decades of use, EG has been the subject of numerous toxicity, metabolism, and pharmacokinetic studies in a variety of species. Although some variability in sensitivity has been observed across species, genders, or strains of animals, these studies have consistently identified the kidney as a primary target organ after acute or chronic exposures (Blood *et al.*, 1962, 1965; Coon *et al.*, 1970; Cruzan *et al.*, 2004; Depass *et al.*, 1986; Dunkelberg, 1987; Gaunt *et al.*, 1974; Hanzlik *et al.*, 1947; Melnick, 1984; Morris *et al.*, 1942; NTP, 1993). In humans, renal toxicity, metabolic acidosis, and central nervous system (CNS) depression, have been the major findings in case reports of intentional or accidental overdosing (Blood, 1965; Eder *et al.*, 1998; Hanzlik *et al.*, 1947; Jacobsen and McMartin, 1986; Morris *et al.*, 1942; Spillane *et al.*, 1991) but not after typical occupational exposures or in controlled exposure studies (Carstens *et al.*, 2003; Gerin *et al.*, 1997; Laitinen *et al.*, 1995; Wills *et al.*, 1974). Ethylene glycol has also been demonstrated to cause developmental effects in rats and mice, but not in rabbits, although at dose levels greater than those associated with kidney toxicity (reviewed in Carney, 1994). This latter effect (developmental toxicity) has been the subject of extensive research interest and regulatory review in recent years (Carney, 1994; CERHR, 2004).

The metabolism of EG and the ability to eliminate metabolites are factors closely associated with its toxicity. Depending on the dose, dose rate, and route of exposure, the main metabolites found in blood, tissues, or excreta in all species studied include unmetabolized EG, CO_2, glycolic acid, and oxalic acid (or calcium oxalate) (Fig. 1). Intermediates such as glycoaldehyde, glyoxal, or glyoxylic acid have not been consistently found *in vivo* in animals or humans, presumably because of their very short half-lives and the presence of more efficient competitive metabolic pathways. The general metabolism of EG appears to be consistent across species, although there are species, strain, and sex differences in the pharmacokinetics of EG and its metabolites that are important

[1] To whom correspondence should be addressed at Biological Monitoring & Modeling, P.O. Box 999, MSIN P7-59 Richland, WA 99352. Fax: (509)376–9064. E-mail: rick.corley@pnl.gov.

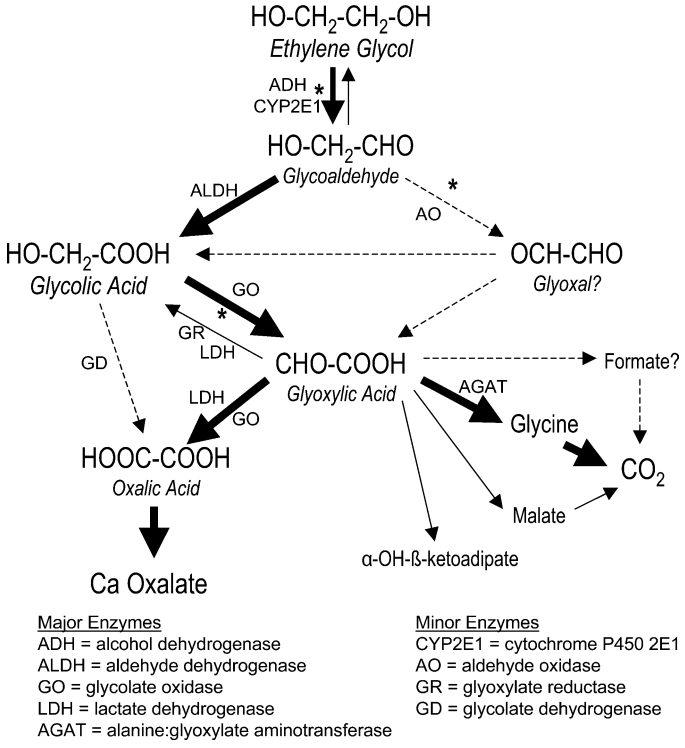

FIG. 1. Metabolism of ethylene glycol (EG) (from: Carstens *et al.,* 2003; Frantz *et al.,* 1996a, 1966b, 1966c; Harris and Richardson, 1980; Marshall, 1982; Pottenger *et al.,* 2001). Steps in the metabolic pathways that are considered slow or rate-limiting are highlighted with an asterisk, and the major pathways leading to known excretory products are highlighted with bold arrows. Pathways with questionable contributions to *in vivo* metabolism in animals or humans are designated with dashed arrows.

in both renal and developmental toxicity (Bowen *et al.*, 1978; Carney, 1994; Carney *et al.*, 1996, 1999; Frantz *et al.*, 1996a, 1996b, 1996c; Introna and Smialek, 1989; Jacobsen *et al.*, 1988; Khera, 1991; Klug *et al.*, 2001; Marshall, 1982).

At high doses, CNS depression has been attributed to the initially high circulating levels of parent EG, whereas metabolic acidosis has been associated with the acid metabolites of EG, most notably glycolic acid (Eder *et al.*, 1998; Jacobsen and McMartin, 1986). Ethylene glycol–induced developmental toxicity is also caused by the intermediate metabolite glycolic acid (GA), which at high dose rates in rats (*i.e.*, $\geq$1000 mg/kg EG by oral gavage) can build up as a result of saturation of metabolism (Carney *et al.*, 1999). Renal damage results in large part from the build-up of one of the terminal metabolites, oxalic acid, which can precipitate with calcium to form crystals. The metabolism of GA to oxalic acid does not appear to occur in the kidneys, indicating that oxalic acid must be transported from the liver (Liao and Richarson, 1972). However, because of the very low solubility of oxalic acid, blood levels of oxalic acid typically don't rise above 10–20 mg/l (0.1–0.2 mM), regardless of dose (Burgess and Drasdo, 1993; Hodgkinson, 1981; Pottenger *et al.*, 2001), which may partially explain the delays observed in renal toxicity after acute exposures.

Throughout its history of use, EG has been the subject of extensive reviews by regulatory agencies to assess potential health risks and establish exposure guidelines. As reviews have progressed (*e.g.*, EPA, 1998; Health Canada, 2001; CERHR, 2004), additional data have been collected on the toxicity, pharmacokinetics, and modes of action of EG developmental and renal toxicity to facilitate the risk assessment processes. Thus, the primary objective of this study was to provide the initial integration of what is now an extensive pharmacokinetic database on EG and one of its important metabolites, GA. Our goal was to create a physiologically based pharmacokinetic (PBPK) model for adult male and female rats and humans to reduce the uncertainties associated with dose, route, and species extrapolations in human health risk assessments. The model includes physiological descriptions and pharmacokinetic data from male and female Sprague-Dawley and Fischer 344 (F344) rats, and male Wistar rats because of their historical uses and differential sensitivities observed in numerous toxicity studies.

To develop this initial model, partition coefficients and metabolic rate constants for EG and GA were determined in rat and human tissues *in vitro*, and the clearances of each metabolite in urine were estimated from applicable *in vivo* studies. The resulting model was then validated against an extensive database in rats and a single human inhalation study, as summarized in Table 1. In a companion study, the initial human model was extended to describe the effect of various treatment regimens and thereby validate the model against several clinical case reports of accidental or intentional overdosing by humans (Corley and McMartin, 2005).

MATERIALS AND METHODS

Model structure and physiological parameters. A diagram of the PBPK model developed using SimuSolv (Registered trademark of The Dow Chemical Company, Midland, MI) to simulate the disposition of EG and GA in adult rats and humans is shown in Figure 2. The model was structured assuming that both EG and GA are distributed "instantaneously" within each tissue (*i.e.*, well-stirred compartments) depending on blood perfusion rates and relative tissue:blood partition coefficients. Although GA has a low pKa (3.83) and is ionized at physiological pH, there is no indication from the *in vivo* kinetics studies of a diffusion limitation to tissue uptake. The EG and GA submodels are linked via saturable metabolism in the liver.

Specific tissues such as the lungs are included in the EG submodel to simulate inhalation exposures; the liver for metabolism; the kidneys as a target organ and excretory organ; skin for subcutaneous injections and eventual simulations of dermal exposures; the gastrointestinal tract for oral absorption; a fat compartment to adjust the body composition and thus tissue distribution of EG and GA for subsequent models of pregnancy and lactation; and the remaining compartments, consisting of tissue groups lumped together on the basis of blood perfusion similarities, to maintain mass balance. An identical structure was used for GA, except that the lung tissues were combined with the composite richly perfused tissue group.

TABLE 1
***In Vivo* Pharmacokinetic Studies Used to Develop (estimate biochemical parameters described in Table 2) and Validate the PBPK Model for Ethylene Glycol (EG) and Glycolic Acid (GA)**

Species/test material route of exposure	Reference
In vivo studies used for model development	
Female Sprague-Dawley rat	
EG oral gavage	Pottenger *et al.* (2001)
Male Wistar rat	
GA oral gavage	Harris and Richardson (1980)
Human	
EG inhalation	Carstens *et al.* (2003)
In vivo studies used for model validation	
Male Sprague-Dawley rat	
EG oral gavage	Lenk *et al.* (1989)
EG oral gavage	Hewlett *et al.* (1989)
Female Sprague-Dawley rat	
EG oral gavage	Sun (1994)
EG oral gavage	Pottenger *et al.* (2001)
Male Wistar rat	
EG & GA oral gavage	Richardson (1973)
EG IP injection	Chou and Richardson (1978)
EG diet	Cruzan *et al.* (2004)
Male F344 rat	
EG IV injection	Marshall (1982)
EG diet	Cruzan *et al.* (2004)
Female F344 rat	
EG IV injection, female F344	Marshall (1982)
Other rat strains	
EG oral gavage, male albino	McChesney *et al.* (1971)
Human	
EG inhalation	Filser (2002)

Anatomical and physiological parameters for rats and humans listed in Table 2, and later in Table 4, were taken from Brown *et al.* (1997), Corley *et al.* (1994), and ICRP (1975). Rat strain–specific renal physiology parameters were taken from Powers (1995). In this adult rat and human model, tissue volumes were scaled linearly with body weight; alveolar ventilation and cardiac output were scaled as (body weight)$^{0.74}$; tissue blood perfusion rates were set as fractions of cardiac output; and glomerular filtration, tubule urine volume, and total urine production were scaled as a fraction of kidney weights.

Equations describing the uptake of EG and GA after inhalation; oral gavage; and dermal, intravenous, or intraperitoneal injection were taken from Andersen *et al.* (1987) and Corley et *al.* (1994). Dietary administration of EG was simulated using a constant rate of uptake (zero order, mg/h) of the total daily dose of EG (mg/kg/day) into gastrointestinal tissues for 12 h of each 24-h day (*i.e.*, 7 pm to 7 am) as a simplified approximation of the nocturnal feeding habits of rats. A pulse function in SimuSolv was used to introduce EG into the gastrointestinal tissues of rats for the first 12 h of each 24-h simulation. A fractional absorption constant was incorporated into dietary uptakes to adjust the bioavailability. Subcutaneous injections or infusions of EG and sodium glycolate were simulated as first-order absorption into venous blood draining the skin compartment.

Although the metabolism of EG appears complex (Fig. 1), the *in vivo* disposition of EG and GA in rats and humans can be described simply by using saturable Michaelis-Menten equations for the two critical rate-limiting steps in the liver: (1) metabolism of EG to GA and (2) metabolism of GA to glyoxylic acid. The elimination of EG into urine was described as a first-order clearance of arterial blood scalable by (body weight)$^{0.70}$. Urinary elimination of GA was also described initially by a similar first-order equation. This simple assumption worked well for many of the high-dose pharmacokinetic studies in the early literature, but it significantly overpredicted the elimination of GA in urine at doses below 200 mg/kg. Therefore an alternative kidney model structure (Fig. 3) was developed, where GA that is filtered by the glomerulus can be reabsorbed from renal tubules by a saturable Michaelis-Menten–like process.

Biochemical parameters. Chemical-specific constants describing absorption, metabolism, blood and tissue partitioning, plasma protein binding, and urinary clearance were (1) measured directly, (2) taken from the literature, or (3) estimated from appropriate studies listed in Table 1 by visually comparing simulations against appropriate data. The maximum likelihood function of SimuSolv was used to compare alternative model structures or parameter estimates as the models were developed and refined. These approaches are described below, with the resulting values and references listed in Tables 3 and 4.

Chemicals. For partition coefficient and plasma protein binding determinations, ethylene glycol (EG; lot no. JR00244CR) and glycolic acid (GA; lot no. 16802LR) were obtained from Sigma-Aldrich. The internal standards D_4-ethylene glycol (lot no. P-6136) and D_2-glycolic acid (lot no. I1-5086) used in gas chromatography/mass spectrometry (GC/MS) analyses were obtained from Cambridge Isotopes Laboratories, Inc. (Andover, MA), and the internal standard, 2-butoxyethanol (lot no. 07847HN) used for gas chromatography/flame ionization detection (GC/FID) analysis of EG was obtained from Sigma-Aldrich. Derivatization agents, N-methyl-t-butyl-silyl-trifluoroacetic acid (lot no. HM12226CU) and tri-n-octyl phosphine (lot no. EH02403) were obtained from Sigma-Aldrich.

For *in vitro* metabolism studies, $^{13}C_2$-ethylene glycol (lot no. 5190), was obtained from Icon Services Inc. (Summit, NJ). This test material was specified to contain a minimum of 99-atom% ^{13}C. Nuclear magnetic resonance (NMR) analysis of this compound afforded a chemical purity of 68.6%, with water as the only identified impurity (31.4%). $^{13}C_2$-GA (lot no. SN0294–1) was obtained from Isotec Inc. (Miamisburg, OH). This test material was specified to contain a minimum of 99-atom% ^{13}C. The NMR analysis of this compound afforded a chemical purity of 83.1%, with 94.3% of the total ^{13}C-labeled components identified as $^{13}C_2$-GA, and four minor ^{13}C-containing impurities observed, comprising 0.7–2.5% each (structures not identified). The remaining non-organic impurities in the $^{13}C_2$-GA test material were assumed to be water and/or inorganic salts. The internal standards D_4-ethylene glycol and unlabeled GA used in GC/MS analyses were obtained from Isotec and Sigma-Aldrich (Milwaukee, WI), respectively. Nicotine adenine dinucleotide (NAD^+), and derivatization agents, pentafluorobenzoyl chloride (PFBCl) and pentafluorobenzyl bromide (PFBBr), were obtained from Sigma-Aldrich. All other compounds and solvents were reagent grade or better.

Test animals. Adult female Sprague-Dawley rats used as blood and tissue donors for partition coefficient and protein binding determinations were purchased from Charles River Laboratories (Portage, MI). Upon arrival at the laboratory, the animals were housed in suspended plastic cages with chipped bedding and acclimated to the laboratory for at least 1 week prior to use. The rooms in which the animals were housed were on a 12-h light cycle (7 am–7 pm) and were designed to maintain adequate temperatures, relative humidity, and airflows for the species under study. Deionized water and Purina Certified Rodent Chow #5002 (Purina Mills, Inc., St. Louis, MO) were provided *ad libitum.*

Partition coefficients. Approximately 11-week-old female Sprague-Dawley rats were anesthetized by CO_2 or i.p. injection with Nembutal (Abbott Laboratories, Abbott Park, IL) and killed by exsanguination to reduce residual tissue blood. Pooled human blood (female) was obtained from a local blood bank. Blood:air partition coefficients for EG were determined using the vial equilibration method of Gargas *et al.* (1989). Heparinized whole blood samples from rats and humans were evaluated undiluted. The concentrations of EG in the headspace of sample and reference vials were determined by gas chromatography/flame ionization detection (GC/FID) as described below for the ultrafiltration method.

Partition coefficients (tissue or blood:saline) were determined for EG and GA in rats from heparinized whole blood, abdominal fat, kidney, liver, lung,

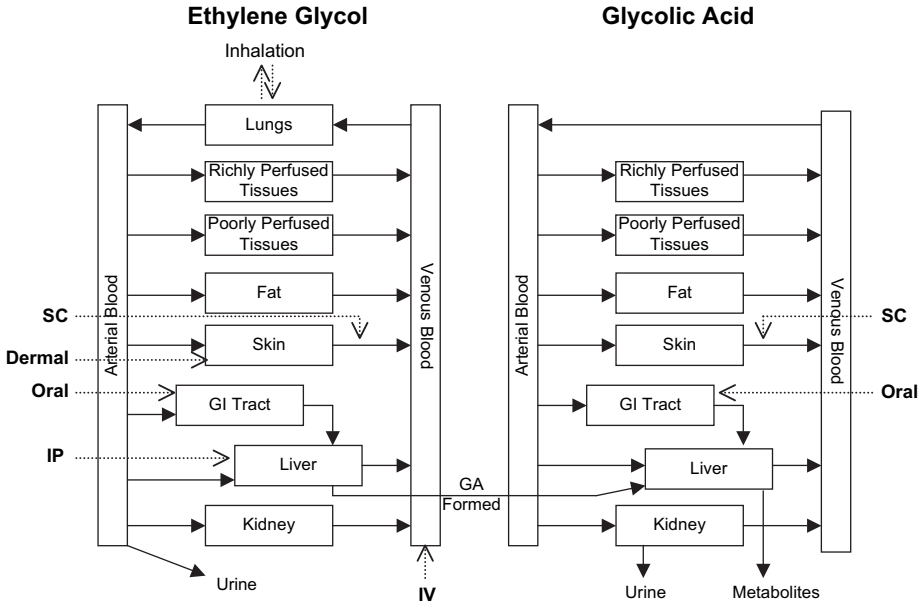

FIG. 2. Physiologically based pharmacokinetic model used to describe the disposition of EG and its metabolite, glycolic acid (GA), in rats and humans after inhalation, oral consumption, intraperitoneal injection (IP), intravenous injection (IV), or subcutaneous infusion (SC) of EG or GA.

brain, thigh muscle, back skin, stomach, small intestine, and cecum according to the methods of Jepson *et al.* (1994) under the following specific conditions: A weighed quantity (300–500 mg) of each tissue or pooled blood was placed in a 20-ml borosilicate glass vial and incubated with 10 ml of either 10 mM EG or GA in phosphate buffered saline (pH 7.4) at 37°C for up to 18 h. Aliquots were taken from each incubation before and after filtration (Amicon MPS micropartition system; Amicon Div., W. R. Grace & Co., Danvers, MA) and analyzed by GC/FID (ethylene glycol) or GC/MS (glycolic acid).

Ethylene glycol was analyzed on a Hewlett Packard 6890 gas chromatograph with flame ionization detection. Separations were achieved with a J&W DB-wax fused silica column (15 m × 0.53 mm id × 1.0 df; J&W Scientific, Folsom, CA). Injections of 1.0 µl (splitless) of plasma, blood, or tissue samples before and after ultrafiltration were spiked with 2-butoxyethanol internal standard (250 µg/g) and injected at 220°C with an initial head pressure of 5 psi (helium) for 2 min, increasing to 10 psi at 20 psi/min. The initial oven temperature was 80°C, increasing to 125°C at 20°C/min, with a final ramp of 30°C/min to 230°C. A Restek 4-mm id cyclo-double gooseneck injection liner was also used.

Glycolic acid was analyzed by GC/MS with a Hewlett Packard 5973 Mass Selective Detector (electron impact ionization) equipped with a Hewlett Packard 6890 Plus gas chromatograph and 7683 autosampler (Hewlett Packard, Avondale, PA). Separations of tri-butylsilyl (TBS) derivatives were achieved with a Restec RTX-5MS fused silica capillary column (30 m × 0.25 mm id, 0.25-µm film thickness; Restec, Bellefonte, PA). Injections (1 µl) were splitless using an unpacked Restec 4-mm id cyclo-double gooseneck liner. Representative chromatography conditions for glycolic acid were as follows: injector temperature was 210°C, the initial oven temperature was 110°C, which was increased at 15°C/min to 200°C, with a final ramp of 25°C/min to 300°C; initial head pressure was a constant 25 psi with helium as the carrier gas. The masses used for quantitation for TBS derivatives of GA were 247 for glycolic acid and 249 for D_2-glycolic acid. Preliminary studies were conducted to determine and correct for nonspecific losses to the filtration system.

Plasma protein binding of glycolic acid. Plasma protein binding can be an important factor in the disposition of small molecular weight weak organic acids. Therefore, protein binding of GA was determined in plasma from female Sprague-Dawley rats and humans by an ultrafiltration method in conjunction with the partition coefficient determinations. Concentrations of GA ranging from 0.01 to 10 mM (rat) or 0.1 to 10 mM (human) were incubated for up to 2 h prior to ultrafiltration in 2 ml samples of plasma gassed with CO_2 to achieve a pH of 7.4 (Amicon MPS micropartition system). Aliquots (100 µl) were taken before and after filtration for analysis by GC/MS. Phenol was also evaluated as a positive control, and preliminary studies were conducted to determine and correct for nonspecific losses of each substrate to the filtration system.

Metabolic rate constants for ethylene glycol. The metabolism of EG has been studied in a variety of *in vitro* systems. Although EG is sequentially metabolized by alcohol and aldehyde dehydrogenases to GA or theoretically via glyoxal (Fig. 1), determination of the rate constants associated with each individual step has been problematic because of analytical difficulties and the lability of aldehyde intermediates. Therefore, apparent metabolic rate constants have been estimated from animal and human tissues or enzyme preparations to describe the formation of GA as a single, rate-limiting step. The parameters associated with this one-step approximation of EG metabolism correspond to the initial rate-limiting step in the metabolism of EG to glycoaldehyde (Fig. 1). Glycoaldehyde is predominately, and very rapidly, converted to GA via aldehyde dehydrogenase; thus, this step can effectively be ignored in this initial PBPK model.

Rajagopal and Ramakrishnan (1994) estimated the Michaelis constant, K_m (11.7 mM), in male Sprague-Dawley rat liver homogenates by measuring the loss of EG. Kukielka and Cederbaum (1991) indirectly determined the apparent K_m (25 mM) and V_{max} (0.36 µmol/h/mg protein) for EG in liver microsomes from male Sprague-Dawley rats by measuring formaldehyde formation. Booth *et al.* (2004) attempted to compare the metabolism of EG by measuring the formation of GA in liver slices from female Sprague-Dawley rats, New Zealand White Rabbits, and human accident victims incubated with EG. Booth *et al.* (2004) were only able to calculate the apparent metabolic rate constants for the rat ($K_m = 23.8$ mM; $V_{max} = 0.164$ µmol/h/mg protein) because little or no GA was detected in rabbit and human liver slices, presumably because of further metabolism. However, these studies generally indicate that the apparent K_m for the metabolism of EG appears to be consistent across species, given the limited data, and it is considerably higher than that reported for ethanol (1–2.7 mM; Blair and Vallee, 1966; Pastino *et al.*, 1997).

The liver slice metabolism data of Booth *et al.* (2004) provided a reasonable starting point for development of the PBPK model. However, simply incorporating the measured K_m values and V_{max} values (adjusted to liver mass

TABLE 2
Physiological Parameters Used in the PBPK Model for Eethylene Glycol in Rats and Humans[a]

Parameter		SD rat[a]	Human
Physiology			
BW	Body weight (kg)[b]	0.23	60
QCC	Cardiac output (l/h/kg)[c]	15	15
QPC	Alveolar ventilation (l/h/kg)	15	15
Tissue volumes (fraction of body weight)[d]			
VBC	Blood	0.059	0.059
VLC	Liver	0.034	0.0314
VKC	Kidneys	0.007	0.0044
VluC	Lungs	0.005	0.0115
VGIC	GI tract	0.05	0.034
VFC	Fat	0.07	0.231
VSKC	Skin	0.19	0.051
VRC	Richly perfused	0.0423	0.0371
VSC	Slowly perfused	0.91 – ∑(other tissues)	
Blood flows (fraction of cardiac output)[d]			
QLC	Liver (includes QGIC)	0.18	0.25
QGIC	GI tract	0.15	0.21
QKC	Kidney	0.141	0.25
QFC	Fat	0.07	0.05
QSKC	Skin	0.058	0.03
QRC	Richly perfused tissues	1.0 – ∑(other tissues)	
QSC	Poorly perfused tissues	0.17	0.17

[a]Initially set up for adult female Sprague-Dawley (SD) rats and humans. Other rat strain-specific parameters are listed in Table 4.
[b]Study specific.
[c]Scalable by $(BW)^{0.74}$.
[d]Brown et *al.* (1997); Corley *et al.* (1997); ICRP (1975).

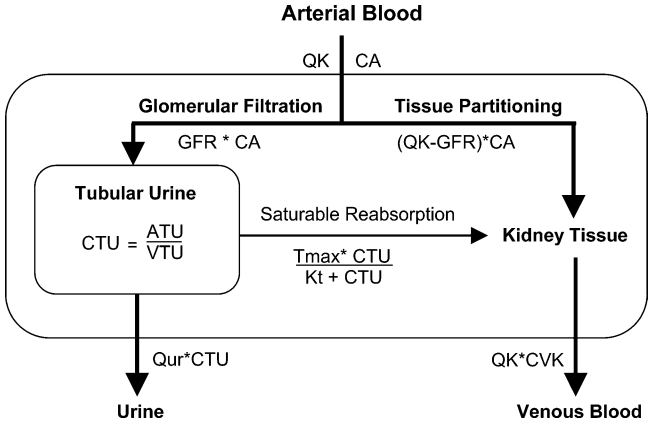

FIG. 3. Structure of the kidney compartment used in the PBPK submodel for GA where QK is the arterial blood flow to kidneys (l/h), CA is arterial blood concentration of GA (mg/l); CVK is the concentration of GA in venous blood draining the kidneys (mg/l); GFR is the glomerular filtration rate (l/h; scaled from the kidney weight); CTU is the concentration of GA in tubule urine (mg/l); ATU is the amount of GA in tubule urine (mg); VTU is the volume of tubule urine (l; calculated as a percentage of the kidney weight); Qur is the urine flow (l/h; scaled to kidney weight); T_{max} is the maximum capacity for saturable reabsorption (mg/h; scaled by $(\text{body weight})^{0.70}$); and Kt is the affinity constant for saturable reabsorption of glycolic acid (mg/l). Values for these parameters are provided in Table 4.

using measured protein yields), led to significant underpredictions of the rates of metabolism *in vivo*. This result was not unexpected because the V_{max} calculated from rat liver slice data was likely an underestimate of the actual rate of GA production because it was likely to be further metabolized as it was formed. Attempts to measure the V_{max} for ethylene glycol more accurately in rat and human liver S9 and cytosolic fractions by measuring the simultaneous disappearance of $^{13}C_2$-ethylene glycol with the glycolate formed were unsuccessful because of the very low yield in metabolism (<1%). Therefore, the apparent K_m (23.8 mM) measured in female Sprague-Dawley rats by Booth *et al.* (2004) was used as the *in vivo* K_m for both rats and humans, and the measured V_{max} values for EG metabolism were increased based upon simulations of the *in vivo* data of Pottenger *et al.* (2001) for non-pregnant female Sprague-Dawley rats and Carstens *et al.* (2003) for humans, and were scaled within species to $(\text{body weight})^{0.7}$.

Metabolic rate constants for glycolic acid. Booth *et al.* (2004) also evaluated the metabolism of glycolic acid in liver slices from female Sprague-Dawley rats, New Zealand White rabbits, and humans by measuring the formation of GA. Because of the degradation in radiochemical purity of the ^{14}C-GA substrate that occurred over the time needed to procure viable human liver samples, only the apparent metabolic rate constants for rats and rabbits were considered for PBPK model development. However, as with EG as a substrate, liver slices maintain all of the enzymes necessary for further metabolism of GA. Therefore, apparent V_{max} may also underestimate the total glycolic acid metabolism. Therefore, additional *in vitro* metabolism studies were conducted using cytosolic fractions isolated from female Sprague-Dawley rats and humans.

Pooled, liver cytosols from female Sprague-Dawley rats (lot 120397C) were obtained from XenoTech LLC (Kansas City, KS). This material contained 25.9 mg protein/ml cytosol. As defined by the vendor, 1 ml of rat liver cytosol was obtained from each 0.539 g of fresh liver tissue. Pooled liver cytosol from 10 female human donors (lot 101998B) was obtained from XenoTech LLC. This material contained 20 mg protein/ml cytosol. According to the vendor, 1 ml of human pooled liver cytosol was estimated to be obtained from each 0.651 g of liver tissue. These protein yields were used to convert *in vitro* metabolic rates (per mg protein) to *in vivo* rates (per mg tissue).

The effects of protein concentrations and cofactor additions were evaluated in preliminary studies to determine optimal incubation conditions. Representative conditions for the incubation of $^{13}C_2$-GA at substrate concentrations of 0.56–11.2 mM with liver cytosol were as follows: liver cytosol (0.05 ml; approximately 1 mg protein) was added to a 4-ml glass vial containing 1.2 ml KH_2PO_4 buffer (0.1 M, pH 7.4) and 0.1 ml EDTA solution (2.9 mM in KH_2PO_4 buffer). Samples were pre-incubated at 37°C for 10 min. Test material, as a stock solution in deionized water, was then added (20 µl), and the samples were incubated at 37°C for 1–24 h. Aliquots (100 µl) of each sample were transferred to 4-ml glass vials, deactivated with 10 µl acetonitrile, and fortified with 1 ml of internal standard solution (25 µg D_4-ethylene glycol + 2.4 µg unlabeled glycolic acid/ml acetone). To each sample was added 0.1 ml 5 N aq. NaOH, 50 µl each PFBCl and PFBBr. Sample aliquots were then vortexed at 50°C for 30 min (Labconco Corporation, Kansas City, MO) to form the diester derivatives of the test materials. The derivatized samples were then diluted with 1.5 ml deionized water and the derivatized analytes were extracted into 1.0 ml toluene (vortex-mixed for approximately 15 s). The toluene layers were isolated after centrifugation at 1278 × g for 10 min and transferred to 2-ml glass GC vials for GC/MS analysis.

The GC/MS analyses were performed on a Hewlett Packard 5989X mass spectrometer (Hewlett Packard, Avondale, PA), equipped with a Hewlett Packard 5890 gas chromatograph and a 7673A autosampler. Separations were achieved in a Phenomenex ZB-5MS fused silica capillary column (30 m × 0.25 mm id × 0.5 µM film; Torrance, CA); helium carrier gas (25 psig) at a flow rate of approximately 1 ml/min; gas chromatograph oven temperature program; 80°C (0.5 min initial hold) to 290°C at 12°/min; injector and capillary transfer line both at 280°C; 1 µl autosampler injection (0.2 min splitless). The mass spectrometer conditions were negative-ion chemical ionization (NCI): ion

TABLE 3
Biochemical Parameters Used in the PBPK Model for Ethylene Glycol (EG) and Glycolic Acid (GA) in Female Sprague-Dawley (SD) Rats and Humans

		EG		GA	
	Parameter	SD Rat	Human	SD Rat	Human
Absorption					
KAS[a]	Oral gavage (h^{-1})	1–5	1	1	1
KSC	Subcutaneous Inj. (h^{-1})	1	—	1	—
KIP	Intraperitoneal Inj. (h^{-1})	1	—	—	—
Distribution (partition coefficients)[b]					
PB	Blood:air	17,901	17,542	—	—
PBS	Blood:saline	1.14	1.14	3.36	3.36
PSKS	Skin:saline	1.36	1.36	2.51	2.51
PSKA	Skin:air[c]	17,901	17,542	—	—
PL	Liver:blood	0.96	0.96	0.97	0.97
PK	Kidney:blood	1.22	1.22	1.40	1.40
PLU	Lung:blood	0.96	0.96	—	—
PF	Fat:blood	0.64	0.64	1.09	1.09
PSK	Skin:blood	1.19	1.19	0.75	0.75
PGI[d]	GI tract:blood	1.48	1.48	0.95	0.95
PR	Richly perfused:blood	0.96	0.96	0.97	0.97
PS[e]	Poorly perfused:blood	0.67	0.67	0.70	0.70
Elimination					
CLC[f]	Urinary clearance (l/h/kg)	0.06	0.06	(0.06)[g]	(0.06)[g]

Note. The model parameters were either estimated independently and held fixed (fixed), measured in independent experiments (measured), or estimated by fitting the model to the data (fitted) as described in the text with the sources for each estimation designated in footnotes to the table.

[a]First-order oral absorption *fixed* to value consistent with other solvents; in some reports, animals were fasted prior to dosing thus *KAS* was increased to 5. Amount absorbed (mg) = Dose – Dose*$e^{-(KAS*T)}$.

[b]Measured in human blood and female Sprague-Dawley rat blood and tissues. Human tissue:blood partition coefficients assumed to be equal to rat (fixed). Partition coefficients assumed to be the same in all rat sexes and strains.

[c]Assumed skin:air same as blood:air.

[d]Average of stomach, small intestines and cecum.

[e]Assumed poorly perfused tissues represented by muscle.

[f]Estimated from female Sprague-Dawley rat data of Pottenger *et al.* (2001).

[g]Alternative model for the first-order clearance of GA from arterial blood scaled from rats by (body weight)$^{0.70}$.

source temperature, 250°C; ionizing current, 0.37 mA; electron energy, 157 eV; methane reagent gas, 2 torr. Quantitation of the diester derivatives of $^{13}C_2$-EG or $^{13}C_2$-GA was achieved by selected ion monitoring at m/z 269 (GA int. std.), 271 ($^{13}C_2$-GA), 452 ($^{13}C_2$-EG), and 454 (D_4- EG int. std.) @ 100–150 ms/ion/scan.

Adjusted substrate concentrations were calculated from the means of S_0 and S_{2h} substrate concentrations, as described by Lee and Wilson (1971). These adjusted substrate concentrations were used in the determination of the kinetic constants K_m and V_{max}. All calculations were performed with Microsoft Excel, v. 97.SR-2, in full precision mode.

Clearance of ethylene glycol and glycolic acid in urine. The elimination of EG and GA in urine was initially simulated as first-order clearances of arterial blood scaled as a function of (body weight)$^{0.7}$. This simple approach worked well for EG over a broad range of doses, but it overpredicted glycolic acid levels in urine of rats at dose levels under 200 mg/kg. To account for the decreased clearances of GA at lower dose levels of EG, the kidney compartment was modified to include glomerular filtration, saturable reabsorption of GA from renal tubules and urine output (Fig. 3). Once the strain-, sex- and species-specific renal physiology parameters were incorporated into the model, parameters for the saturable reabsorption of GA from renal tubules (assuming Michaelis-Menten kinetics) were estimated from the data of Pottenger *et al.* (2001) for female Sprague-Dawley rats, Harris and Richardson (1980) for male Wistar rats, and Carstens *et al.* (2003) for male humans, using the maximum likelihood estimate function of SimuSolv and the values reported in Table 4.

RESULTS

Model Development

Partition coefficients. As expected from the physical/chemical properties of ethylene glycol, the blood:air partition coefficients determined by head-space analyses were very high (17,901 in rats and 17,542 in humans). Thus, uptake by inhalation is expected to be ventilation-limited. Tissue:blood partition coefficients for rat tissues were all generally within of 40% of unity for both ethylene glycol and glycolic acid, indicating that the distribution of these chemicals will be relatively uniform in the body. These results are similar to other low molecular weight alcohols and their acid metabolites (Clarke *et al.*, 1993; Corley *et al.*, 1994; Horton *et al.*, 1992; Pastino *et al.*, 1997). Blood:air and tissue:blood partition coefficients for female Sprague-Dawley rats and humans are provided in Table 3. These partition coefficients were used to simulate the distribution of EG and GA in male and female Sprague-Dawley, F344, and Wistar rats, and in humans.

Plasma protein binding. Essentially no binding of glycolic acid to proteins was detected in plasma from female Sprague-Dawley rats over a concentration range of 0.01–10 mM. Low levels of saturable plasma protein binding were detected in human plasma (13% at 0.1 mM, the lowest concentration used, and 2% at 10 mM). The positive control, phenol (60 μg/ml), was bound at expected levels of ~40% in rat plasma and 48% in human plasma (Liao and Oehme, 1981) under identical assay conditions. Given the very low (human) or absent (rat) levels of protein binding detected in plasma, no equations for protein binding were included in the PBPK model.

Metabolism and urinary clearance of ethylene glycol. Pottenger *et al.* (2001) conducted an extensive pharmacokinetic study with $^{13}C_2$-ethylene glycol using a broad range of oral gavage doses (10–2500 mg/kg) in pregnant (gestation day 10) and non-pregnant female Sprague-Dawley rats and modern GC/MS methods for analysis of ethylene glycol, glycolic acid, and oxalic acid. In their study, Pottenger *et al.* demonstrated that the kinetics of EG and GA in blood and urine are unaffected by pregnancy status, at least over a 24-h period starting on gestational day 10, which is during the critical window of susceptibility to developmental toxicity as reviewed by Carney (1994). Thus, these data were used in

TABLE 4
Renal Physiology and Biochemical Parameters and Liver Metabolism Parameters for Rats and Humans

							Metabolism parameters			
	Renal parameters (GA)						EG		GA	
Species	BW[a] (kg)	VKC[a] (l/kg BW)	GFRC[a] (l/h/kg)	QURC[a] (l/h/kg)	KT[b] (mg/l)	$T_{max}EC$[b] (mg/h/kg)	KM1[c] (mg/l)	$V_{max}1C$[d] (mg/h/kg)	KM2[c] (mg/l)	$V_{max}2C$[c] (mg/h/kg)
Female SD	0.280	0.007279	41.04	0.174	840	20	1479	290	60	44.3
Male SD	0.425	0.006524	62.1	0.174	840	20	1479	290	60	44.3
Female F344	0.160	0.007235	26.4	0.277	840	20	1479	290	60	44.3
Male F344	0.260	0.006349	54.4	0.119	840	20	1479	290	60	44.3
Male Wistar	0.460	0.006156	58.3	0.331	616	15	1479	290	60	44.3
Human (male)	70	0.00443	24.19	0.212	840	14	1479	1300	14.4	107.6
Human (female)	58	0.00474	27.28	0.152	840	14	1479	1300	14.4	107.6

Note: The model parameters were either estimated independently and held fixed (*fixed*), measured in independent experiments (measured), or estimated by fitting the model to the data (fitted) as described in the text,with the sources for each estimation designated in footnotes to the table. The volume of urine in renal tubules (VTUC, scaled to kidney weight) was arbitrarily fixed to 10% of the kidney volume in each strain, sex, or species.

[a]Body weights (BW) for young adult (~15 weeks of age for rats), kidney volume (VKC, scaled fraction of BW), glomerular filtration rate (GFRC, scaled fraction of kidney weight), and urine flow (QURC, scaled fraction of kidney weight) measured by Powers (1995) for rats and by ICRP (1975) or Guyton (1976) for humans.

[b]Michaelis constant (KT) and maximum capacity ($T_{max}EC$) for tubule reabsorption of glycolic acid fitted to pregnant Sprague-Dawley (SD) rat data of Pottenger *et al.* (2001), male Wistar rat data of Harris and Richardson (1980), and human data of Carstens *et al.* (2003). $T_{max}EC$ is scaled by $(\text{body weight})^{0.70}$. Parameters were held fixed for male SD rat and male and female F344 rats and female humans.

[c]Measured in female SD rat liver slice by Booth *et al.* (2003) for EG and female SD rat and female human liver cytosols for GA and held fixed for other strains or sexes.

[d]Estimated $V_{max}C$ for EG metabolism in female SD rats from Pottenger *et al.* (2001) and held fixed for other rat strains; estimated for male humans from Carstens *et al.* (2003), and held fixed for females. Each study-specific V_{max} was calculated from $V_{max}C*(BW)^{0.70}$.

developing and validating estimates of the V_{max} for metabolism of EG (after setting the apparent K_m to that of Booth *et al.*, 2004) and the renal clearances of both EG and GA, once the metabolism and tissue partition parameters were established as described in the methods section.

The V_{max} for EG metabolism and the first-order clearance of EG were estimated through repeated iterations of simulations and visual comparisons with the concentrations of EG and GA in blood and cumulative amounts of EG eliminated in urine. The first-order clearance of EG, scaled by $(\text{body weight})^{0.70}$, estimated from the data of Pottenger *et al.* (2001) was used for all strains of rats and for humans in all subsequent simulations. Results from these simulations are shown in Figure 4, and final parameter estimates are given in Tables 3 and 4.

Although the *in vivo* V_{max} for the metabolism of EG was significantly increased from the *in vitro* determination to simulate the kinetics of EG in rats, it was still insufficient to describe the metabolism of EG in a controlled human exposure study where two volunteers inhaled $^{13}C_2$-EG injected into a vaporization flask every 15 min over 4 h (Carstens *et al.*, 2003). Simulations were conducted for each individual assuming the exposures were conducted at steady-state time-weighted average concentrations of 20 or 27 ppm based on total amounts inhaled (1.34 or 1.43 mmol) and the body weight–dependent ventilation rates, cardiac outputs, and biochemical constants shown in Tables 2–4. Using the K_m for the metabolism of EG and the urinary clearance of EG determined in rats, the V_{max} for the metabolism of EG in humans was increased from 290 to 1300 mg/kg/h to fit the data of Carstens *et al.* (2003), as shown in Figure 5. The resulting simulations provided a good description of individual differences in the concentrations of EG and GA in plasma and the cumulative excretion of GA in urine. There appeared to be a greater variability between subjects in the cumulative urinary clearance of EG, although the model was consistent with the

TABLE 5
Calculations of the Margins of Safety Between Simulated Peak Blood Concentrations of Glycolic Acid in Humans after the Maximum Tolerated Inhalation Exposure Regimens of Wills *et al.* (1974) and the 2 mM Threshold Estimated by Carney *et al.* (2002) for Developmental Toxicity in Rats

Aerosol concentration (mg/m^3)	Exposure duration (min)	C_{max} GA in Blood (μM)	Ratio to 2 mM threshold
188	15	0.361	5,540
244	2	0.0611	32,733
308	2 breaths	0.0011	869,565

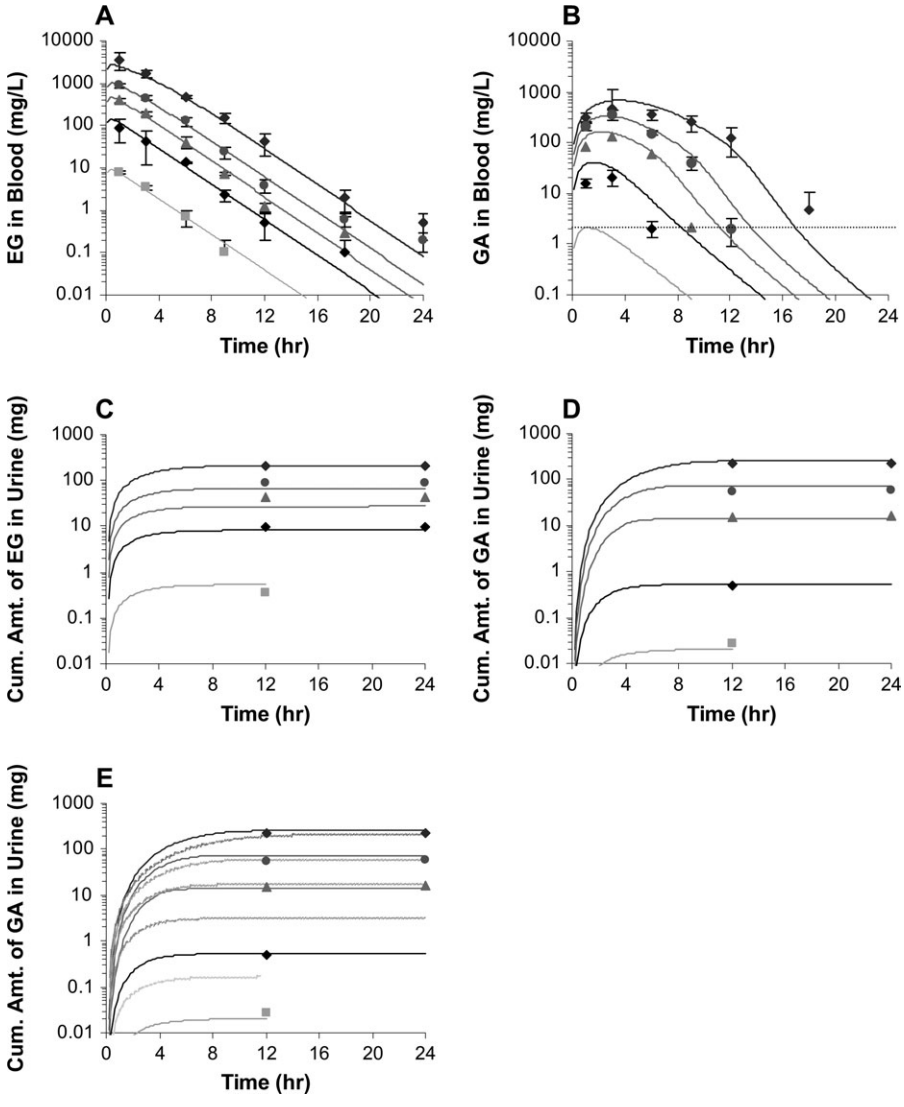

FIG. 4. Data (symbols) and model calibrations (lines) of the (a) concentration of ethylene glycol (EG) in blood; (b) concentration of glycolic acid (GA) in blood; (c) cumulative amounts of EG eliminated in the urine; and (d) cumulative amounts of GA eliminated in the urine of pregnant female Sprague-Dawley rats administered EG by oral gavage at 10, 150, 500, 1000, or 2500 mg/kg on gestational day 10 (data from Pottenger *et al.*, 2001). In (e), simulations of the cumulative amounts of GA eliminated in the urine are shown, using the final saturable reabsorption model (solid lines) vs. the alternative first-order clearance model (dotted lines). The levels of GA in blood after a 10 mg/kg oral dose of EG were all below the limits of quantitation (2.1 mg/l; see dotted line). Data were used to estimate V_{max} for metabolism of ethylene glycol and parameters for renal clearance of ethylene glycol and saturable renal absorption of GA for female Sprague-Dawley rats.

average of the two subjects by simply scaling the physiology and biochemical constants by each subject's body weight.

Metabolism and urinary clearance of glycolic acid. The apparent K_m values for the metabolism of GA determined from female Sprague-Dawley rat and female human cytosols were calculated to be 0.79 and 0.19 mM, respectively. These values are in the range of those reported previously for liver slices from rats and humans (0.28 and 0.43 mM, respectively, Booth *et al.*, 2004) and purified glycolate oxidase enzymes isolated from rat liver (0.2 mM, McGroaty *et al*. 1974; 2.1 mM, Kun *et al*. 1954) and human liver (0.34 mM, Fry *et al*. 1979). The V_{max} values calculated for GA metabolism in liver cytosol were 0.54 and 0.41 μmoles/h/mg protein, respectively, for rats and humans, which are, as expected, considerably higher than those reported in liver slices by Booth *et al.* (2004), presumably because of the ability of the liver slices to further metabolize the measured product, glyoxylic acid.

There was some potential non-linearity in Lineweaver-Burke plots of the rates of metabolism of GA at various substrate concentrations (data not shown), especially in human liver cytosol. Such non-linearities may be a reflection of GA oxidation by more than one hepatic enzyme or isoform. Glycolate oxidase has been shown to exist as two isozymes

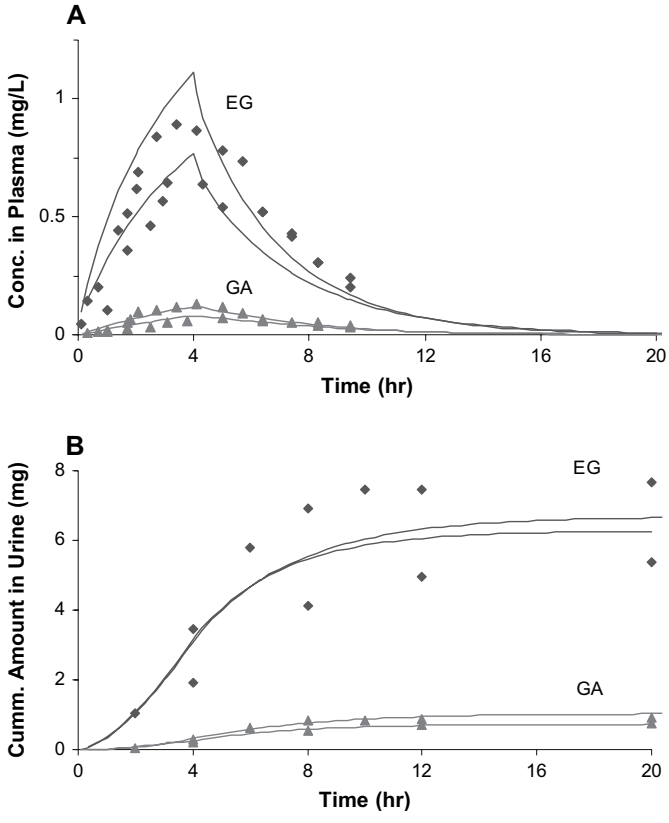

FIG. 5. Data (symbols) and model calibrations (lines) of the (a) concentration of ethylene glycol (EG) and glycolic acid (GA) in blood and (b) cumulative amounts of EG and GA eliminated in the urine of two male human volunteers exposed by inhalation to $^{13}C_2$-EG (data from Carstens *et al.*, 2003). Data were used to estimate the V_{max} for metabolism of EG and the saturable renal reabsorption of GA.

(Duley and Holmes, 1976). As a result, the K_m and V_{max} parameters in the current study should be defined as apparent values. The apparent K_m and V_{max} values determined in this study were used directly in the PBPK model after normalizing cytosolic protein yields to whole liver weight (Table 4).

Once the EG metabolism and clearance and GA metabolism parameters were obtained, the parameters for the clearance of GA in female Sprague-Dawley rats were estimated from the data of Pottenger *et al.* (2001) by repeated iterations of simulations and visual comparisons with the concentrations of GA in blood and cumulative amounts of EG eliminated in the urine.

As discussed previously, using a first-order clearance process for urinary excretion of GA provided good fits to the high-dose groups used by Pottenger *et al.* (2001), as well as to the other high-dose kinetic studies summarized below (simulations shown in Fig. 4e), but it failed to describe the kinetics of GA in blood and urine at the two lower dose levels (10 and 150 mg/kg). At these two lower dose levels, the measured amounts of GA eliminated in the urine were significantly less than that predicted by a linear excretion process, suggesting that GA was likely being reabsorbed from the renal tubules, as are many other small molecular weight amino acids or other essential nutrients. Because the first-order process worked well for the higher doses in this and other studies (from 500 to as high as 5000 mg/kg), it is likely that reabsorption was a saturable process. Thus, the alternative model structure shown in Figure 3 was used in place of a simple first-order clearance process, and the data of Pottenger *et al.* (2001) were used to estimate the Michaelis-Menten constants associated with renal tubule reabsorption. Final estimates of the parameters associated with the urinary clearance of GA in female Sprague-Dawley rats are summarized in Tables 3 and 4. Simulations of the concentrations of GA in blood and cumulative amounts excreted in the urine of the pregnant female Sprague-Dawley rats are shown in Figure 4.

Male Wistar rats appear to be unusually susceptible among rat strains to the renal toxicity of EG after subchronic and chronic dietary administration (Cruzan et al., 2004; DePass *et al.*, 1986; Gaunt *et al.*, 1974). This susceptibility may be explained, at least partially, by differences in the kinetics of EG metabolites, most notably oxalic acid (Cruzan *et al.*, 2004) and its precursor, GA (Harris and Richardson, 1980; Richardson, 1973). Harris and Richardson (1980) determined the dose-dependent conversion of glycolate to glyoxylate, oxalic acid, and CO_2, as well as the elimination of glycolate in urine in

male Wistar rats administered sodium glycolate by oral gavage. Using the glycolate metabolism rate constants derived from female Sprague-Dawley rats, the urinary clearance parameters were estimated from the data of Harris and Richardson, with the results provided in Table 4 and the simulations shown in Figure 6.

For humans, the data of Carstens *et al.* (2003) were used to estimate the parameters associated with the renal clearance of GA. From human renal physiology (Table 4) and metabolism rate constants determined *in vitro*, the parameters associated with the renal clearance of GA were determined by visual comparisons with the concentrations of GA in plasma (Fig. 5a) and the cumulative amounts of GA eliminated in the urine (Fig. 5b) with the values reported in Table 4.

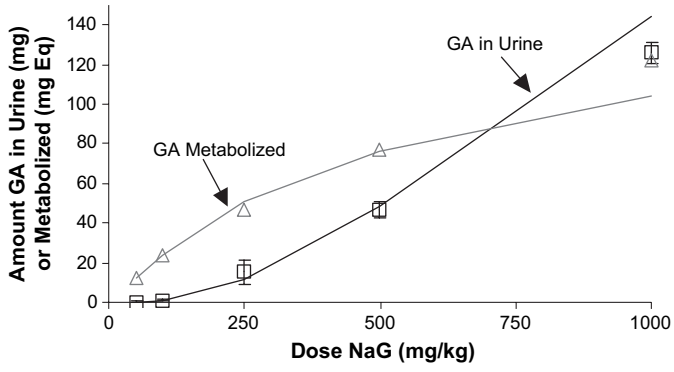

FIG. 6. Data (symbols) and model calibrations (lines) for the total amounts of GA eliminated in the urine or metabolized after oral doses of 50, 100, 250, 500, or 1000 mg/kg sodium glycolate in male Wistar rats (data from Harris and Richardson, 1980). Data were used to estimate parameters for saturable renal reabsorption of GA for male Wistar rats.

Model Validation

There are numerous metabolism and pharmacokinetic studies in a variety of strains of rats. Although simulations have been conducted with all available data sets, those studies that provided information on the kinetics of both EG and GA were considered the most important for model development and validation. Data sets from studies that were not used in the development of model parameter estimates were used to validate the PBPK model (Table 1). In these cases, only the appropriate sex-, strain-, or species-specific biology, scaled according to experiment-specific body weights and exposure regimens, were changed in the model to perform each simulation. If the simulations were in reasonable agreement with the new data (generally within a factor of two of the measured values and/or with similar kinetic behavior over the uptake, distribution, and clearance phases), the model was considered ''validated.'' For the few cases where agreement was not achieved, as was the case with the simulations of GA clearance in urine at low doses, plausible explanations for the disparity were presented or, just as importantly, insights into underlying biochemical behaviors were postulated and an alternate model structure was proposed.

Because of the large number of studies used in the validation of the PBPK model for rats, only representative results are presented. The remaining data and simulations used to validate the PBPK model are provided as Supplementary Material online and include simulations of data from Pottenger *et al.* (2001), Lenk *et al.* (1989), Hewlett *et al.* (1989), Richardson (1973), Marshall (1982), McChesney *et al.* (1971), and Cruzan *et al.* (2004).

Female Sprague-Dawley rat data of Sun (1994). Female Sprague-Dawley rats were administered ^{14}C-ethylene glycol at either 10 or 1000 mg/kg by oral gavage (Sun, 1994). Simulations of the concentrations of EG and GA, assuming that plasma is equivalent to whole blood, are shown in Figure 7. The model provided a reasonable description of the concentrations of EG at both dose levels and of GA at the highest dose, but it significantly overpredicted the concentrations in plasma at the lowest dose. This lowest dose level, 10 mg/kg, was also used in the Pottenger *et al.* (2001) study, where the concentrations of glycolic acid were below the limits of reliable quantitation (2.1 mg/l). In the Sun (1994) study, GA was determined by high-performance liquid chromatography (HPLC) with refractive index and radiodetection; thus, much lower levels (0.01 mg/l) were detected. Because there were no corresponding data in urine, it is possible that the parameters for renal tubule reabsorption of GA derived from the data of Pottenger *et al.* (2001) result in a higher rate of reabsorption than warranted. However, a reduction in the reabsorption of GA would result in a reduced ability to fit the Pottenger *et al.* (2001) data, which reflect both the blood and urine levels of GA over a comparable dose range (10–2500 mg/kg).

Male Wistar rat data of Chou and Richardson (1978). Chou and Richardson (1978) administered a high dose (2700 mg/kg) of ^{14}C-EG by i.p. injection to five male Wistar rats. The rats were sacrificed at 2-h intervals post-dosing, and their data, along with simulations of the concentrations of EG and GA in plasma, are shown in Figure 8. The model provided an excellent simulation of the EG concentrations. However, the concentrations of GA appeared to be similar in the five rats sacrificed over a 10-h period (concentrations remained close to 750 mg/l) whereas the model indicated that the levels should increase to approximately 600 mg/l by 4 h and then slowly decline to under 200 mg/l by 10 h. It is possible that the analysis of GA was confounded by interference, as quantitation was based solely on liquid scintillation counting of specific fractions eluting from a liquid chromatography column, with no confirmation (*e.g.*, GC/MS) of the identity.

Human data from Filser (2002). In preliminary studies conducted to develop and validate methods for the analysis of EG and its metabolites in plasma and urine (see Carstens *et al.*, 2003), a single human volunteer was exposed for 4 h to 30 ppm

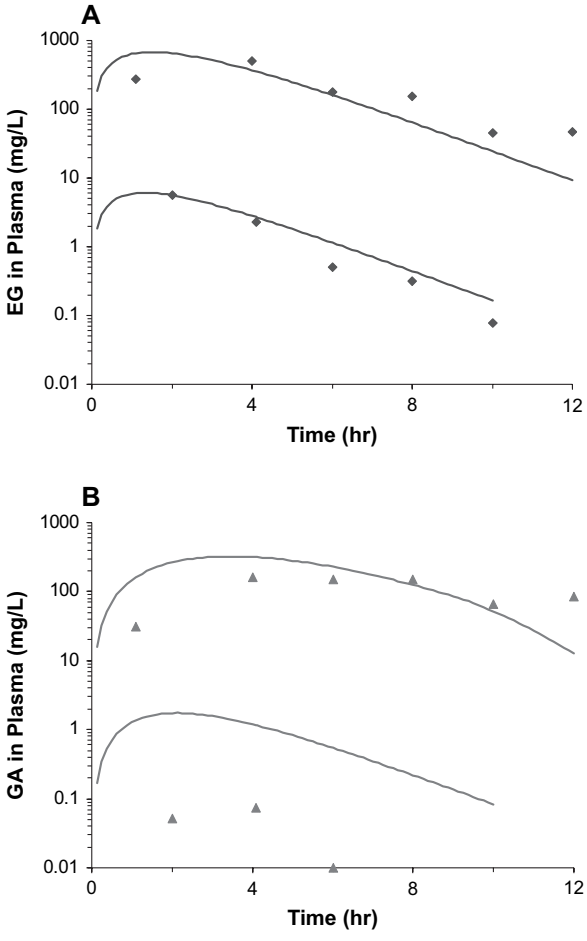

FIG. 7. Data (symbols) and model validations (lines) of the concentrations (a) ethylene glycol and (b) glycolic acid in blood of female Sprague-Dawley rats administered either 10 or 1000 mg/kg EG by oral gavage (data from Sun, 1994).

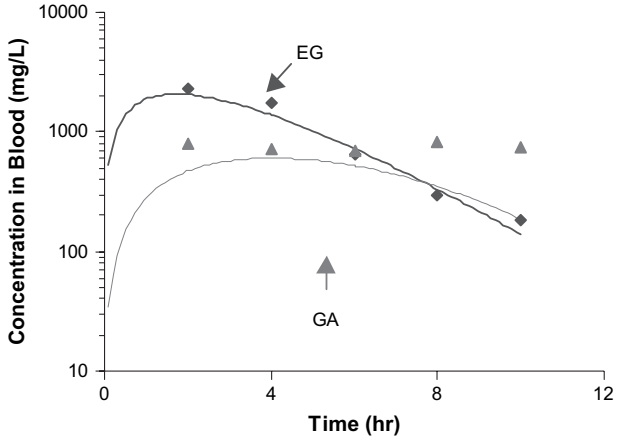

FIG. 8. Data (symbols) and model validations (lines) of the concentrations EG and GA in blood of male Wistar rats administered 2700 mg/kg EG by i.p. injection (data from Chou and Richardson, 1978). Each data point represents one of five rats sacrificed at 2, 4, 6, 8, or 10 h after injection.

EG by inhalation (Filser, 2002). Simulations of the total amounts of EG and GA eliminated in this volunteer are shown in Figure 9. These data provide a partial validation of the human PBPK model. However, this low exposure was below that which causes saturation in any of the processes involved in the metabolism and elimination of EG and its metabolites. Additional validation simulations, where humans ingested large quantities of EG either intentionally or accidentally, that result in saturation of metabolism or renal tubule reabsorption are included in a companion paper (Corley and McMartin, 2005).

DISCUSSION

A PBPK model was developed to integrate the extensive database on the kinetics and mode of action for EG and one of its metabolites, glycolic acid, in male and female Sprague-Dawley, F344, and Wistar rats, and in humans. The developmentally toxic metabolite, glycolic acid, is a minor metabolite at low doses of EG, but it can become a major metabolite at bolus oral doses of 200–1000 mg/kg in rodents as a result of metabolic saturation. Pottenger *et al.* (2001) have shown that the kinetics of EG and GA in blood and urine are unaffected by pregnancy, at least on gestational days 10–11 during the critical window of susceptibility. Thus, this initial model, which describes the kinetics of EG and GA in male and female (pregnant and non-pregnant) rats and human volunteers, represents the first step toward interspecies comparisons of maternal blood levels of this developmentally important metabolite.

In this model, the metabolism of EG to GA was described as a single saturable, rate-limiting step. This simplification was justified based on the lack of data to either parameterize or validate the individual steps in the metabolism of EG to GA (both the ability to measure the individual steps shown in Figure 1 and the lack of detection of glycoaldehyde or glyoxal *in vivo*). Furthermore, differences associated with isoforms of ADH and ALDH, which are important for describing the kinetics of ethanol, have not been clear enough to warrant segregation of these steps based upon the available animal and human case report data for ethylene glycol (see Corley and McMartin, 2005).

The renal clearance of GA in urine was also described by means of a more physiologically realistic model than simple use of an empirical, first-order clearance term, like that used for EG. One of the advantages of this approach is that species-, sex-, and strain-specific urine production parameters, which could be important to the toxicity of terminal metabolites of EG, such as oxalic acid, could be used. Furthermore, it was possible to compensate for the measured increases in urine output (osmotic diuresis) that occurred during the subchronic dietary toxicity/pharmacokinetic study of Cruzan *et al.* (2004; see Supplementary Material online) in a physiologically meaningful way. This osmotic diuresis, presumably caused by high

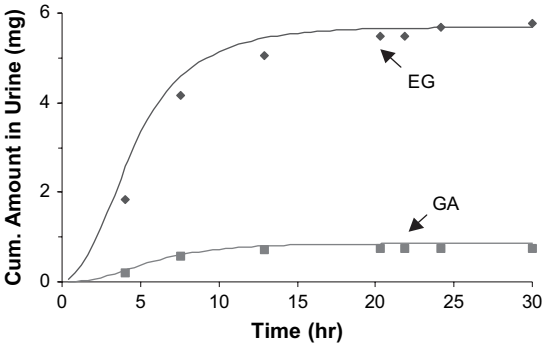

FIG. 9. Data (symbols) and model validations (lines) of the cumulative amounts of EG and GA eliminated in the urine of a human volunteer exposed for 4 h to 30 ppm EG (data from Carstens *et al.*, 2003).

concentrations of EG in tubule urine (a high percentage of the dose is excreted unchanged), did not affect filtration, but it did influence the reabsorption of GA, as it must compete with urine output. Thus, while the number of model parameters may increase over the use of simple, first-order clearance terms, such parameters had to be supported by experimental measurements. This support effectively reduces the overall uncertainties in the PBPK model and focuses attention on areas where fewer data are available (*i.e.*, the effect of renal toxicity on urinary clearance mechanisms).

Although a more complete description of the kinetics of EG and GA in the developing rat embryo is under way, there are currently no approaches available, either ethically or technically, that can be used to validate such models for EG in the developing human embryo (Corley *et al.*, 2003). Thus, it is likely that extrapolations of internal dose surrogates for human health risks associated with developmental toxicity will be limited to maternal blood levels of GA as described below. As a result, the current PBPK model described in this article was extended to simulate the kinetics of EG and GA in controlled human pharmacokinetic studies conducted at low doses or exposures and using human-specific physiology, blood:air partition coefficients, and metabolic rate constants coupled with biochemical parameters scaled from rats.

As discussed in the companion article (Corley and McMartin, 2005), there are numerous case reports in the literature where large quantities of EG, generally as the primary constituent in antifreeze, have been consumed intentionally or accidentally by men, women, or children. In several of these case reports, blood or urine samples were collected and analyzed for EG, and occasionally for GA as well. Although the actual dose levels, timing of blood samples from consumption, effect of other constituents consumed (drugs, alcohol), impact of toxicity, and the various treatment regimens employed by hospitals, all may contribute to alterations in the pharmacokinetics of EG and GA, the model was structured to accommodate several of these effects in a biologically meaningful manner. Thus, the human case

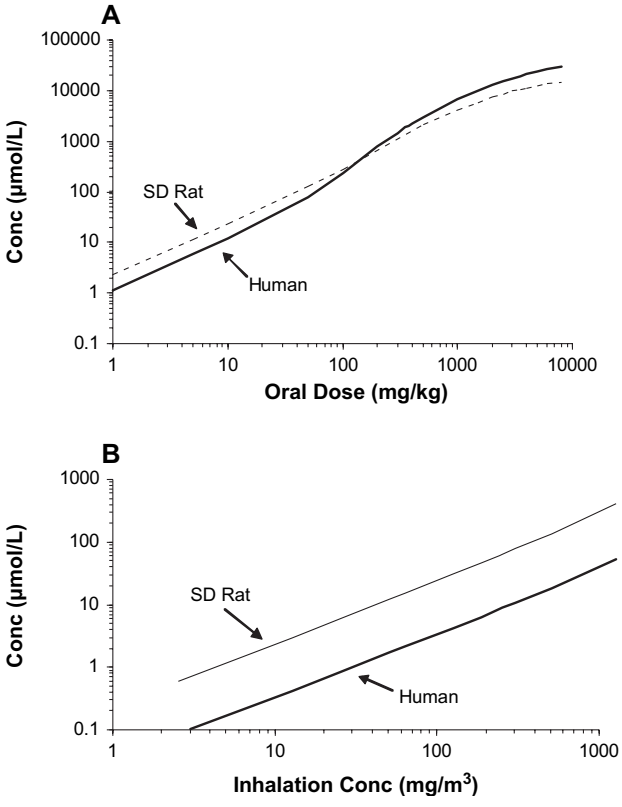

FIG. 10. A dose–response comparison of internal dose surrogates (C_{max} for GA in blood) in female Sprague-Dawley rats, male Wistar Rats, male F344 rats, and humans after either (a) oral or (b) inhalation exposures to EG (note that the theoretical maximum vapor concentration is ~200 mg/m^3 at 20°C). Simulations were conducted using body weights for young adult rats (15 weeks of age; female S-D, 280 g; male F344, 260 g; male Wistar, 460 g), and young adult human (70 kg male and 58 kg female).

report data served to validate the current human PBPK model developed from *in vitro* and controlled *in vivo* studies for high dose/exposure scenarios as well as the controlled, low dose/exposures described herein.

Thus, internal dose surrogates that could potentially be included in developmental toxicity risk assessments were compared across dose, route of exposure, and species. Potential dose surrogates consisted of either the maximum concentration (C_{max}) or the area under the curve (AUC) for GA in blood. Both of these dose surrogates have their strengths and weaknesses. Of the two choices, C_{max} offers the advantage of being highly sensitive to dose rate, which according to Carney *et al.* (1999) is critical to the developmental toxicity of GA. One of the potential disadvantages to using C_{max} is its sensitivity to the first-order absorption rate constant, *KAS*. Fortunately, in this, and the companion study of Corley and McMartin (2005), *KAS* was remarkably consistent across numerous rat and human oral studies and case reports, which effectively reduces the concern for using this internal dose surrogate. Thus, the C_{max} simulations for GA in blood after either oral bolus dosing or 6-h inhalation exposures were conducted for male and female

humans, female Sprague-Dawley rats, and male F344 and Wistar rats (for simplification, only the results from the female Sprague-Dawley rat and female human are shown in Figure 10a). At oral bolus dose levels <150 mg/kg, higher C_{max} values of GA in blood are predicted in all of the rat strains than in humans. However, higher C_{max} levels are predicted in humans at higher dose levels with a rank order of human male > human female > female SD rat > male F344 rat > male Wistar rat, primarily because of the saturation in metabolism of glycolic acid. The species and strain differences are primarily associated with differences in rates of metabolism and urinary clearance of GA. Similar results were observed if the AUC for GA was used as a dose surrogate (simulations not shown).

After inhalation exposures, however, where species differences in respiratory rates and cardiac output become more important (as compared with bolus oral dosing), the C_{max} levels for GA are significantly higher in rats than in humans (Fig. 10b). For occupational exposures, this route of exposure is more important than bolus oral dosing.

Given the low volatility of EG (0.06 mm Hg at 20°C), the theoretical maximum vapor concentration for ethylene glycol is only ~79 ppm (~200 mg/m^3). This low volatility, coupled with the potential irritancy of EG aerosols, effectively limits the possibility for developmental toxicity in humans after inhalation exposures to EG. For example, Wills *et al.* (1974) conducted a controlled human inhalation exposure study at various maximum tolerated inhalation concentrations of EG aerosol. At 188 mg/m^3, exposures were conducted for only 15 min. At higher levels, volunteers tolerated only 2 min of exposure to 244 mg/m^3 or two breaths of exposure to 308 mg/m^3. Simulations were conducted for each of these exposure scenarios to predict the C_{max} for GA in blood and to calculate the margin of safety between peak blood levels of GA and the 2 mM threshold determined by Carney *et al.* (2002) for developmental effects in rats, as shown in Table 5. Based on these simulations, it is unlikely that humans can inhale enough EG to cause developmental effects. Also, humans could only achieve the 2 mM threshold (determined in rats) if they consumed bolus oral doses >350 mg/kg (>20 g EG for a 58 kg female) during the critical window of susceptibility based on simulations of peak (C_{max}) blood concentrations of GA.

At present the data available are insufficient to extend the PBPK model to describe oxalic acid and calcium oxalate dosimetry in the kidneys of rats and humans. Nearly all published kinetic studies are limited to EG and, in several cases, GA analysis. Analyses of oxalic acid, if performed, are often complicated by endogenous production of oxalic acid or dietary contributions, inadequate analytical sensitivity or specificity, or are limited to just a few urine samples. Only recently have studies been conducted that include adequate controls for comparison of the utilization of ^{13}C-labeled test materials to differentiated metabolites from administered EG derived from endogenous or dietary sources (*e.g.*, Carstens *et al.*, 2003; Cruzan *et al.*, 2004; Pottenger *et al.*, 2001). Similar studies are in progress to determine the concentrations of EG, GA, and oxalic acid in blood, urine, and kidneys of male Wistar rats exposed to EG in their diets for up to 1 year. To construct a model for oxalic acid, it would be important to determine the relative rates of metabolism of glyoxylic acid to oxalic acid versus the production of glycine and other metabolites across species (see Fig. 1). It may also be important to understand calcium regulation to describe the impact of long-term EG exposure on calcium oxalate crystal formation. Nevertheless, this model represents the first biologically based integration of a significant database on the kinetics of EG that can be used to quantitatively link exposure to the first key metabolite associated with metabolic acidosis and developmental toxicity, glycolic acid. Further validation of the human model can be found in the companion study of Corley and McMartin (2005).

SUPPLEMENTARY MATERIAL

Supplementary data are available online at www.toxsci.oupjournals.org.

ACKNOWLEDGMENTS

This study was funded by the Ethylene Glycol Panel, American Chemistry Council. Two co-authors (M.J.B. and E.W.C.) are employees of one of the ACC member companies. The lead author (R.A.C.) serves as a consultant to ACC under a contract with Battelle Memorial Institute. The authors certify that all research involving human subjects was done under full compliance with all government policies and the Helsinki Declaration.

REFERENCES

Andersen, M. E., Clewell, H. J., III, Gargas, M. L., Smith, F. A., and Reitz, R. H. (1987). Physiologically based pharmacokinetics and the risk assessment process for methylene chloride. *Toxicol. Appl. Pharmacol.* **87,** 185–205.

Blair, A. H., and Vallee, B. L. (1966). Some catalytic properties of human liver alcohol dehydrogenase. *Biochemistry* **5,** 2026–2034.

Blood, F. R. (1965). Chronic toxicity of ethylene glycol in the rat. *Fundam. Cosmet. Toxicol.* **3,** 229–234.

Blood, F. R., Elliott, G. A., and Wright, M. S. (1962). Chronic toxicity of ethylene glycol in the monkey. *Toxicol. Appl. Pharmacol.* **4,** 489–491.

Booth, E. D., Dofferhoff, O., Boogaard, P. J., and Watson, W. P. (2004). Comparison of the metabolism of ethylene glycol and glycolic acid in vitro by precision-cut tissue slices from female rat, rabbit and human liver. *Xenobiotica* **34,** 31–48.

Bowen, D. A. L., Minty, P. S. B., and Sengupta, A. (1978). Two fatal cases of ethylene glycol poisoning. *Med. Sci. Law* **18,** 102–107.

Brown, R. P., Delp, M. D., Lindstedt, S. L., Rhomberg, L. R., and Beliles, R. P. (1997) Physiological parameter values for physiologically based pharmacokinetic models. *Toxicol. Ind. Hith.* **13,** 407–484.

Burgess, J., and Drasdo, D. N. (1993). Solubilities of calcium salts of dicarboxylic acids in methanol-water mixtures; transfer chemical potentials of dicarboxylate anions. *Polyhedron* **12,** 2905–2911.

Carney, E. W. (1994). An integrated perspective on the developmental toxicity of ethylene glycol. *Reprod. Toxicol.* **8,** 99–113.

Carney, E. W., Freshour, N. L., Dittenber, D. L., and Dryzga, M. D. (1999). Ethylene glycol developmental toxicity: Unraveling the roles of glycolic acid and metabolic acidosis. *Toxicol. Sci.* **50,** 117–126.

Carney, E. W., Liberacki, A. B., Bartels, M. J., and Breslin, W. J. (1996). Identification of proximate toxicant for ethylene glycol developmental toxicity using rat whole embryo culture. *Teratology* **53,** 38–46.

Carney, E. W., Liberacki, A. B., Tornesi, B., and Markham, D. A. (2002). Ethylene glycol: Effect of Dose-Rate on Developmental Toxicity. R&D Report of The Dow Chemical Co., Midland, MI.

Carstens, J., Csanady, G. A., Faller, T. H., and Filser, J. G. (2003). Human inhalation exposure to ethylene glycol. *Arch. Toxicol.* **77,** 425–432.

Center for the Evaluation of Risks to Human Reproduction (CERHR). (2004). NTP-CERHR Expert panel report on the reproductive and developmental toxicity of ethylene glycol. NIH Publication No. 04-4481. January, 2004 (http://cerhr.niehs.nih.gov)

Chou, J. Y., and Richardson, K. E. (1978). The effect of pyrazole on ethylene glycol toxicity and metabolism in the rat. *Toxicol. Appl. Pharmacol.* **43,** 33–44.

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.

Coon, R. A., Jones, R. A., Jenkins, L. J., Jr., and Siegel, J. (1970). Animal inhalation studies on ammonia, ethylene glycol, formaldehyde, dimethylamine and ethanol. *Toxicol. Appl. Pharmacol.* **16,** 646–655.

Corley, R. A., and McMartin, K. E. (2005). Incorporation of therapeutic interventions in physiologically based pharmacokinetic modeling of human clinical case reports of accidental or intentional overdosing with ethylene glycol. *Toxicol. Sci.* in press.

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.

Corley, R. A., Mast, T. J., Carney, E. W., Rogers, J. M., and Daston, G. P. (2003). Physiologically based modeling of pregnancy and lactation: Improving dose–response assessments in children's health risk assessments. *CRC Crit. Rev. Toxicol.* **33(2),** 137–211.

Cruzan, G., Corley, R. A., Hard, G. C., Mertens, J. W. M., McMartin, K. E., Snellings, W. B., Gingell, R. and Deyo, J. A. (2004). Subchronic toxicity of ethylene glycol in male Wistar and F344 rats is related to metabolism and clearance of metabolites. *Toxicol. Sci.* **81,** 502–511.

DePass, L. R., Garman, R. H., Woodside, M. D., Giddens, W. E., Maronpot, R. R., and Weil, C. S. (1986). Chronic toxicity and oncogenicity studies of ethylene glycol in rats and mice. *Fund. Appl. Toxicol.* **7,** 547–565.

Duley, J. A., and Holmes, R. S. (1976). L-α-hydroxyacid oxidase isozymes. *Eur. J. Biochem.* **63,** 163–173.

Dunkelberg, H. (1987). Carcinogenic activity of ethylene oxide and its reaction products 2-chloroethanol, 2-bromoethanol, ethylene glycol and diethylene glycol. III. Testing of ethylene glycol and diethylene glycol for carcinogenicity. *Zentralbl. Bakteriol. Hyg. B.* **183,** 358–365.

Eder, A. F., McGrath, C. M., Dowdy, Y. G., Tomaszewski, J. E., Rosenberg, F. M., Wilson, R. B., Wolf, B. A., and Shaw, L. M. (1998). Ethylene glycol poisoning: Toxicokinetic and analytical factors affecting laboratory diagnosis. *Clin. Chem.* **44,** 168–177.

Environmental Protection Agency (EPA). (1998). Testing consent order and export notification requirements for ethylene glycol. *Fed. Reg.* **63,** 15130–15133.

Filser, J. G. (2002). Human inhalation exposure to ethylene glycol. Interim Progress Report to CEFIC. January 2002.

Frantz, S. W., Beskitt, J. L., Grosse, C. M., Tallant, M. J., Dietz F. K., and Ballantyne, B. (1996a). Pharmacokinetics of ethylene glycol. I. Plasma disposition after single intravenous, peroral, or percutaneous doses in female Sprague-Dawley rats and CD-1 mice. *Drug Metab. Dispos*. **24,** 911–921.

Frantz, S. W., Beskitt, J. L., Grosse, C. M., Tallant, M. J., Dietz, F. K., and Ballantyne, B. (1996b). Pharmacokinetics of ethylene glycol. II. Tissue distribution, dose-dependent elimination, and identification of urinary metabolites following single intravenous, peroral or percutaneous doses in the female Sprague-Dawley rat and CD-1 mouse. *Xenobiotica* **26,** 1195–1220.

Frantz, S. W., Beskitt, J. L., Grosse, C. M., Tallant, M. J., Zourelias, L. A., and Ballantyne, B. (1996c). Pharmacokinetics of ethylene glycol. III. Plasma disposition and metabolic fate after single increasing intravenous, peroral or percutaneous doses in male Sprague-Dawley rats. *Xenobiotica* **26,** 515–539.

Fray, D. W., and Richardson, K. E. (1979). Isolation and characterization of glycolic acid oxidase from human liver. *Biochim. Biophys. Acta* **568,** 135–144.

Gargas, M. L., Burgess, R. J., Voisoard, D. E., Cason, G. H., and Andersen, M. E. (1989). Partition coefficients of low-molecular-weight volatile chemicals in various liquids and tissues. *Toxicol. Appl. Pharmacol.* **98,** 87–99.

Gaunt, I. F., Hardy, J., Gangolli, S. D., Butterworth, K. R., and Lloyd, A. G. (1974). *Short-Term Toxicity of Monoethylene Glycol in the Rat.* BIBRA International, Carshalton, Surrey, U.K. Research Report 4/1974.

Gerin, M., Patrice, S., Begin, D., Goldberg, M. S., Vyskocil, A., Adib, G., Drolet, D., and Viau, C. (1997). A study of ethylene glycol exposure and kidney function of aircraft de-icing workers. *Int. Arch. Occup. Environ. Health* **69,** 255–265.

Guyton, A. C. (1976). *Textbook of Medical Physiology.* W. B. Saunders Company, Philadelphia, pg. 444.

Hanzlik, P. H., Lawrence, W. S., and Laquer, G. L. (1947). Comparative chronic toxicity of diethylene glycol monoethyl ether (Carbitol) and some related glycols: Results of continued drinking and feeding. *J. Ind. Hyg. Toxicol.* **29,** 233–241.

Harris, K. S., and Richardson, K. E. (1980). Glycolate in the diet and its conversion to urinary oxalate in the rat. *Invest. Urol.* **18,** 106–109.

Health Canada. (2001). Notice of suspension of five-year period for ethylene glycol, a substance specified on the Priority Substance List (subsection 78(2) of the Canadian Environmental Protection Act, 1999). *Canada Gazette Part I* **134,** 3548–3549.

Hodgkison, A. (1981). Sampling errors in the determination of urine calcium and oxalate: solubility of calcium oxalate in HCI-urine mixtures. *Clin. Chem. Acta* **109,** 239–244.

Horton, V. L., Higuchi, M. A., and Rickert, D. E. (1992). Physiologically based pharmacokinetic model for methanol in rats, monkeys and humans. *Toxicol. Appl. Pharmacol.* **117,** 26–36.

International Commission on Radiation Protection (ICRP). (1975). Reference Man: Anatomical, Physiological and Metabolic Characteristics. Report of the task group on Reference Man (Snyder, W. S., Cook, M. J., Nasset, E. S., Karhausen, L. R., Howells, G. P., and Tipton, I. H., Eds.). ICRP Publication 23, Elsevier, New York, pp. 354.

Introna, F., and Smialek, J. E. (1989). Antifreeze (ethylene glycol) in toxications in Baltimore. Report of six cases. *Acta Morphologica Hungarica* **37,** 245–263.

Jacobsen, D., Hewlett, T. P., Webb, R., Brown, S. T., Ordinario, A. T., and McMartin, K. E. (1988). Ethylene glycol intoxication: Evaluation of kinetics and crystalluria. *Am. J. Med.* **84,** 145–152.

Jacobsen, D., Ovrebo, S., Ostborg, J., and Sejersted, O. M. (1984). Glycolate causes the acidosis in ethylene glycol poisoning and is effectively removed by haemodialysis. *Acta Med. Scand.* **216,** 409–416.

Jepson, G. W., Haover, D. K., Black, R. K., McCafferty, J. D., Mahle, D. A., and Gearhart, J. M. (1994). A partion coefficient determination method for nonvolatile chemicals in biological tissues. *Toxicol. Appl. Pharmacol.* **22,** 519–524.

Khera, K. S. (1991). Chemically induced alterations in maternal homeostasis and histology of conceptus: Their etiologic significance in rat fetal anomalies. *Teratology* **44,** 259–297.

Klug, S., Merker, H.-J., and Jackh, R. (2001). Effects of ethylene glycol and metabolites on *in vitro* development of rat embryos during organogenesis. *Toxicol. In Vitro* **6,** 635–642.

Kukielka, E., and Cederbaum, A. I. (1991). Oxidation of ethylene glycol to formaldehyde by rat liver microsomes. Role of cytochrome P-450 and reactive oxygen species. *Drug Metab. Disp.* **19,** 1108–1114.

Kun, E., DeChary, J. M., and Pitot, H. C. (1954). The oxidation of glycolic acid by a liver enzyme. *J. Biol. Chem.* **210,** 269–280.

Laitinen J., Liesivuori, J., and Savolainen, H. (1995). Exposure to glycols and their renal effects in motor servicing workers. *Occup. Med.* **45,** 259–262.

Lee, J.-H., and Wilson, R. B. (1971). Enzymic parameters: Measurement of V_{max} and K_m. *Biochim. Biophys. Acta* **242,** 519–522.

Lenk, W., Lohr, D., and Sonnenbichler, J. (1989). Pharmacokinetics and biotransformation of diethylene glycol and ethylene glycol in the rat. *Xenobiotica* **19,** 961–979.

Liao, L. L., and Richardson, K. E. (1972). The metabolism of oxalate precursors in isolated perfused rat livers. *Arch. Biochem. Biophys.* **153,** 438–448.

Liao, T. H., and Oehme, F. W. (1981). Plasma protein binding of phenol in dogs and rats as determined by equilibrium dialysis and ultrafiltration. *Toxicol. Appl. Pharmacol.* **57,** 226–230.

Marshall, T. C., and Y. S. Cheng. (1983). Deposition and fate of inhaled ethylene glycol vapor and condensation aerosol in the rat. *Fundam. Appl. Toxicol.* **3,** 175–181.

McChesney, E., Goldberg, L., Parekh, C. K., Russell, J. C., and Min, B. H. (1971). Reappraisal of the toxicology of ethylene glycol. 2: Metabolism studies in laboratory animals. *Food Cosmet. Toxicol.* **9,** 21–38.

McGroaty, E., Berlin. H., Wied, D. M., Robert, G., and Tolbert, N. E. (1974). Alpha hydroxy acid oxidation by peroxisomes. *Arch. Biochem. Biophys.* **161,** 194–210.

Melnick, R. L. (1984). Toxicities of ethylene glycol and ethylene glycol monethyl ether in F344/N rats and B6C3F1 mice. *Environ. Health Perspect.* **57,** 147–155.

Morris, H. J., Nelson, A. A., and Calvery, H. O. (1942). Observations on the chronic toxicities of propylene glycol, ethylene glycol, diethylene glycol, ethylene glycol mono-ethyl ether, and diethylene glycol mono-ethyl ether. *J. Pharmacol. Exp. Ther.* **74,** 266–273.

NTP (National Toxicology Program). (1993). NTP technical report on the toxicology and carcinogenesis studies of ethylene glycol (CAS Nos. 107–21–1) in B6C3F1 mice (feed studies). National Toxicology Program, US Department of Health and Human Services. NIH Publication 93–3144.

Pastino, G. M., Asgharian, B., Roberts, K., Medinsky, M. A., and Bond, J. A. (1997). A comparison of physiologically based pharmacokinetic model predictions and experimental data for inhaled ethanol in male and female B6C3F1 mice, F344 rats, and humans. *Toxicol. Appl. Pharmacol.* **145,** 147–157.

Pottenger, L. H., Carney, E. W., and Bartels, M. J. (2001). Dose-dependent nonlinear pharmacokinetics of ethylene glycol metabolites in pregnant (GD10) and nonpregnant Sprague-Dawley rats following oral administration of ethylene glycol. *Toxicol. Sci.* **62,** 10–19.

Powers, W. J. (1995). Renal toxicology: Renal function parameters for adult Fischer-344, Sprague-Dawley, and Wistar rats. In *CRC Handbook of Toxicology* (Derelanko, M. J., and Hollinger, M. A., Eds.) CRC Press, Boca Raton, FL, pp. 317–336.

Rajagopal, G., and Ramakrishnan, S. (1994). Hepatic metabolism of ethylene glycol (E6) and its relevance to ethanol as antidote in E6 toxicity. *Ind. S. Pharmacol.* **26,** 108–111.

Richardson, K. E. (1973). The effect of partial hepatectomy on the toxicity of ethylene glycol, glycolic acid, glyoxylic acid and glycine. *Toxicol. Appl. Pharmacol.* **24,** 530–538.

SRI. (2003). *Chemical Economics Handbook (CEH) Product Review: Mono-, Di- and Triethylene Glycols.* SRI International, Menlo Park, CA. November 2003.

Sun, J. D. (1994). Identification of ethylene glycol metabolites in the plasma of female Sprague-Dawley rats and CD-1 mice. R&D Report of the Union Carbide Corporation, Export, PA.

Wills, J. H., Coulston, F., Harris, E. S., McChesney, E. W., Russell, J. C., and Serrone, D. M. (1974). Inhalation of aerosolized ethylene glycol by man. *Clin. Toxicol*. **7,** 463–476.