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January 20, 2003

Dr. Michael Shelby
Director, CERHR
NIEHS
79 T.W., Alexander Dr.
Bldg. 4401, Rm. 103
PO Box 12233, MD EC-32
Research Triangle Park, NC 27709

RE:

Dear Dr. Shelby:

Thank you for the opportunity to present the results from our research on the toxicokinetics of ethylene glycol at your expert panel meeting on February 11-13, 2003. I am currently a Staff Scientist in The Chemical Dosimetry Group, Battelle Northwest. Our research group specializes in the conduct of *in vitro* and *in vivo* metabolism and pharmacokinetic studies and the development of physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) models for use in human health risk assessments. Our research is supported from grants from NIH, NIOSH, EPA, DOE or contracts from U.S. and European chemical industries.

As suggested in the draft NTP-CERHR Expert Panel Report on the Reproductive and Developmental Toxicity of Ethylene Glycol (December, 2002), there are data that would be useful in the development of a PBPK model for ethylene glycol. We wholeheartedly agree with this suggestion and believe that such a model would be invaluable in the risk assessment process. Since 1995, we have had the opportunity to conduct several pharmacokinetic and mode of action studies for the Ethylene Glycol Panel of the American Chemistry Council. The ultimate goal of this research is to develop a PBPK model that would improve the dosimetry component of human health risk assessments (Corley et al., 2000; Corley, 2000). With the recent completion of a human pharmacokinetic study (Carstens et al., 2002), we extended the model from rat to the human and evaluated its performance against several high dose human case reports in the literature. In the few minutes that I have available during your upcoming expert panel meeting, I will present some of the key results from this project. In particular, I will focus on an integrated comparison of the kinetics of the developmentally toxic metabolite, glycolic acid, in rats vs. humans under a variety of exposure scenarios. An important outcome of this comparison is the realization that there is little or no risk to human development under reasonably foreseeable human exposure conditions that would occur under normal use of ethylene glycol or materials containing ethylene glycol.

For example, the low volatility of ethylene glycol, coupled with the irritant properties of ethylene glycol aerosols, reduces the potential for significant human exposures by the inhalation route. At exposure levels that are within established occupational exposure limits, the blood levels of glycolic acid would not be expected to be significantly different from levels produced via endogenous metabolism. Furthermore, even if humans could withstand high concentrations of aerosols (Wills et al. (1974) indicates that concentrations >300 mg/m^3 are intolerable), they would not likely achieve blood levels of glycolic acid that approach the 2 mM threshold established by Carney et al. (2000) for developmental toxicity in a rat. Based upon the PBPK analysis, such high blood levels are only predicted for humans following high bolus oral doses in excess of 500 mg/kg body weight, which approaches the lethal dose range.

In well-documented human case reports, the primary effects of high lethal or near lethal oral doses involve central nervous system depression, metabolic acidosis and, if not effectively treated, renal toxicity or renal failure. The latter effect (renal toxicity) is attributable to one of the terminal metabolites of ethylene glycol, oxalic acid, which at high doses can precipitate in renal tissues and urine as the poorly soluble salt, calcium oxalate. Since oxalic acid does not play a role in developmental or reproductive toxicity, I will limit the presentation of our research to the PBPK model for ethylene glycol and glycolic acid. In the attachments to this letter, I have included background information on the studies involved in the development and validation of the PBPK model (presentation of the rat PBPK model at the 2000 Society of Toxicology meeting); the recent extension of the rat PBPK model to the human which was made possible by the work of Dr. Filser's laboratory; and simulations of maternal blood levels of glycolic acid in rats and humans following oral and inhalation exposures from a recent report on strain and species differences in the pharmacokinetics of ethylene glycol.

I look forward to presenting this brief overview in February and the potential interactions with your expert panel.

Best regards,

Richard A. Corley, Ph.D.
The Chemical Dosimetry Group

Encl.

cc: W. Gulledge, ACC
J. Moore, SI
A. Iannucchi, SI

ATTACHMENT #1
DEVELOPMENT OF A PBPK MODEL FOR ETHYLENE GLYCOL AND ITS METABOLITE, GLYCOLIC ACID

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Battelle, Pacific Northwest Laboratory, Richland, WA, USA

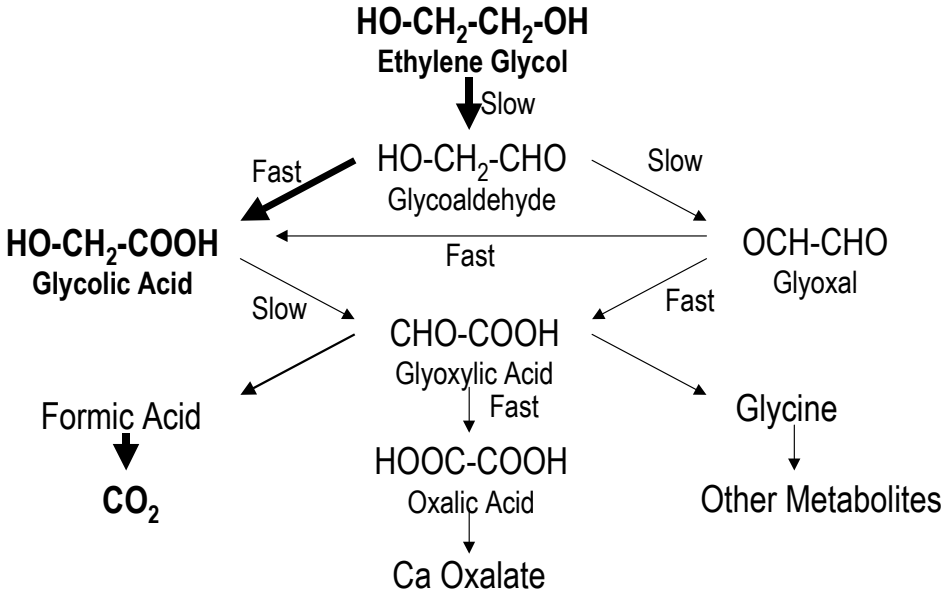
Presented at the 39th Annual Meeting of The Society of Toxicology
March 19-23, 2000, Philadelphia, PA. Abstract #442.

ABSTRACT

Ethylene Glycol (EG) is a major industrial chemical. An extensive database has been amassed on EG's toxicity and modes of action. At high oral bolus doses (≥500 mg/kg/day), EG causes renal and developmental toxicity. EG's toxicity has been primarily attributed to its major metabolite, glycolic acid (GA), which shows a high degree of dose-, route- and species-dependency. A physiologically based pharmacokinetic (PBPK) model was developed to describe the disposition of EG and GA in female rats, including pregnancy. Metabolic rate constants for EG and GA were estimated from liver slice kinetic studies. Partition coefficients for EG and GA were determined by vial equilibration and ultrafiltration methods. The PBPK model included inhalation, oral, dermal, intravenous and subcutaneous routes of administration. Metabolism of EG and GA were described in the liver with elimination via the kidneys. Several rat metabolism studies were simulated. Pregnancy had no effect on maternal EG and GA kinetics over a broad dose range. Simulations were consistent with studies indicating that metabolism of EG to GA was essentially first-order (linear) up to 2500 mg/kg/day while the metabolism of GA saturated between 200 and 1000 mg/kg/day. This resulted in non-linear increases in blood GA concentrations, which correlate with the toxicity of EG. (Sponsored by the Ethylene Glycol Panel of the American Chemistry Council).

INTRODUCTION

- **Commercial applications of ethylene glycol (EG)**
  - solvent in paints and inks
  - hydraulic fluids
  - antifreeze
- **Several EG metabolites also constituents of diets, metabolites of water disinfection byproducts or products of endogenous biosynthesis**
- **Large gavage doses (≥500 mg/kg/day) can cause systemic and developmental toxicity in rats and mice (Carney et al., 1999)**
- **Metabolism and pharmacokinetics are key to toxicity**



  - Metabolism to glycolic acid (GA) critical determinant for developmental toxicity in rats and mice (Carney et al., 1996; Carney et al., 1999)
    - At low doses (20-200 mg/kg)
      - GA is a minor metabolite (<5%)
      - CO_2 is a major metabolite (~30-40%)
    - At high doses (200-2000 mg/kg)
      - GA is a major metabolite (20-50%)
      - CO_2 is reduced (<25%)
    - High GA levels result in metabolic acidosis
  - Oxalic acid, a terminal metabolite, accounts for <2% of the dose
  - Other metabolites have very short half-lives and are difficult to detect

  - Bioavailability in rats and mice (Frantz et al., 1996; Marshall and Cheng, 1983)
    - Oral > Inhalation > Dermal

- **Driving forces for PBPK model development**
  - Dose-, route- and species-dependency in GA kinetics, consistent with toxicity
  - Exposure assessment guidelines (e.g. RfC/RfD) encourage the use of validated PBPK models in risk assessments

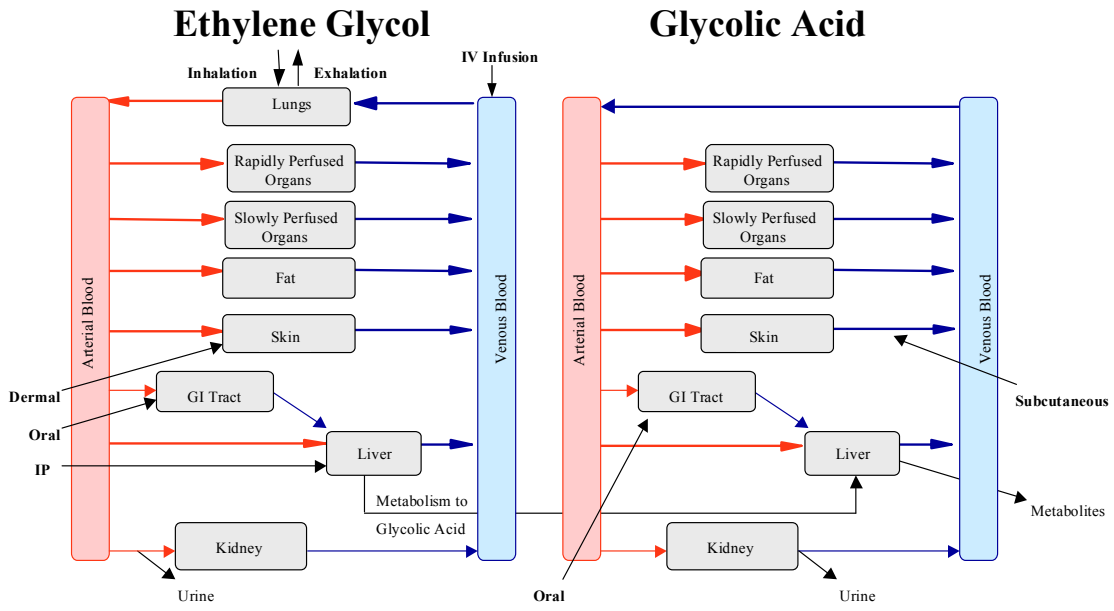
OBJECTIVES

As part of an integrated, multi-laboratory research program on the mechanism of action of EG's developmental toxicity and target tissue dosimetry in human health risk assessments, the objectives of this study were to:

- **Develop an initial PBPK model for ethylene glycol and its major metabolite, glycolic acid, in rats**
- **Compare/validate the PBPK model against existing literature and identify data gaps**
- **Utilize the PBPK model to design future studies**

MATERIALS AND METHODS

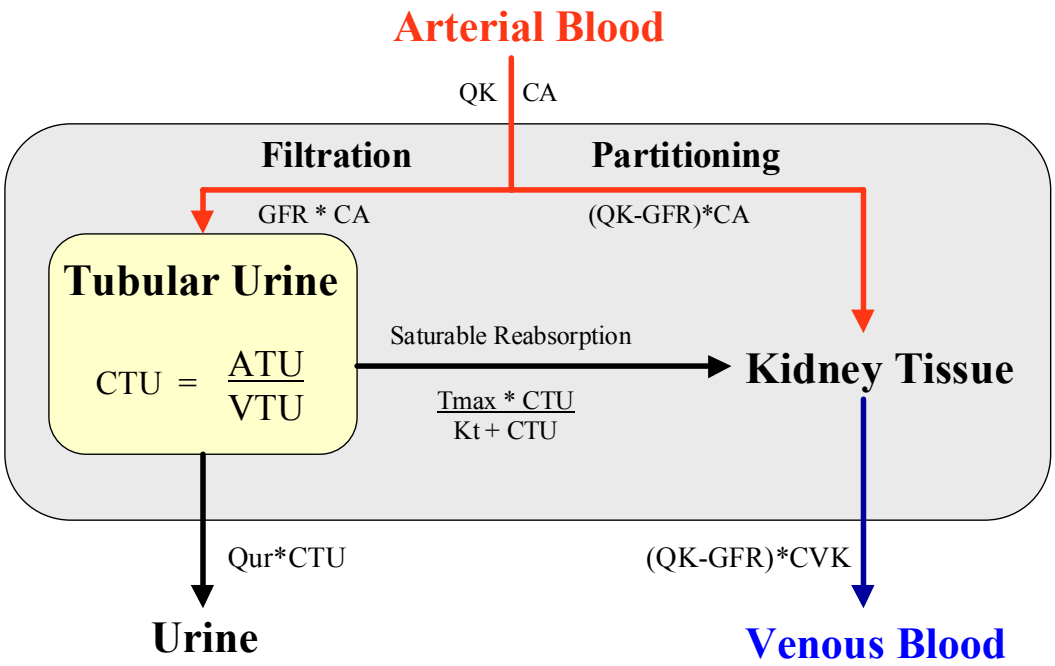
PBPK MODEL STRUCTURE AND ASSUMPTIONS



- **Routes of exposure**
  - Oral gavage (1st -order absorption into GI compartment)
  - Intraperitoneal injection (1st-order absorption into liver compartment)
  - Intravenous infusion (direct input into venous blood)
  - Subcutaneous injection (1st-order absorption into venous blood draining skin)
  - Inhalation (vapor)
  - Dermal (format of Jepson & McDougal, 1997)
- **Metabolism in the liver**
  - First-order metabolism of EG to GA
  - Saturable metabolism of GA
  - Competitive inhibition of GA metabolism by EG (format of Tardif et al., 1997)
    - Reduced metabolism of GA at high dose levels (>3 g/kg) not accounted for by saturable metabolism

- **Renal clearance**
  - First-order clearance from arterial blood for EG
  - Higher clearance of GA into urine observed at doses >1000 mg/kg
    - Higher clearance not associated with saturation in plasma protein binding (no significant binding detected in ultrafiltration studies)
    - Non-linear clearance of GA was described using a kidney model incorporating
      - Glomerular filtration (GFR) of GA into tubular urine
      - Saturable reabsorption of GA from tubular urine

GA KIDNEY MODEL



PARAMETER ESTIMATION

- **Partition coefficients**
  - EG blood:air measured by vial equilibration (Gargas et al., 1989)
  - EG and GA blood:saline and tissue:saline measured by ultrafiltration (Jepson et al., 1994 as modified by Corley et al., 1994)
- **Plasma protein binding**
  - GA plasma protein binding by ultrafiltration (Morgott and Dryzga, 1986)
- **Metabolism**
  - Use of rate constants from *in vitro* liver slice studies (Booth and Watson, 1999a) resulted in significant underpredictions of EG metabolism to GA *in vivo*; thus:
    - EG metabolism estimated from *in vivo* kinetics in non-pregnant female SD rats (Pottenger et al., 1998)
  - GA metabolism determined from *in vitro* liver slice studies in female SD rats (Booth and Watson, 1999b)
- **Renal clearance**
  - EG clearance estimated from *in vivo* kinetics in non-pregnant female SD rats (Pottenger et al., 1998)
  - GA clearance estimated from *in vivo* kinetics of GA in male Wistar rats (Richardson, 1973; Harris & Richardson, 1980) and non-pregnant female SD rats (Pottenger et al., 1998)

- **Model validation studies currently utilized**

Rat Strain, Sex	Administered Compound	Route of Administration	Reference
F344, Male and Female	EG	IV	Marshall (1982)
Wistar, Male	EG	IP	Chou & Richardson (1978)
Not specified, Male	EG	Gavage	McChesney et al. (1971)
Wistar, Male	EG	Gavage	Richardson (1973)
SD, Male	EG	Gavage	Hewlett et al. (1989)
SD, Male	EG	Gavage	Lenk et al. (1989)
SD, Female	EG	Gavage	Pottenger et al. (1998)
SD, Pregnant Female	EG	Gavage	Carney et al. (1997a and b)
SD, Pregnant Female	EG	Gavage	Pottenger et al. (1998)
SD, Pregnant Female	GA	Gavage	Carney et al. (1997a)
SD, Pregnant Female	NaG[a]	SC	Carney et al. (1997a)
Wistar, Male	NaG	Gavage	Richardson (1973)

[a]NaG = sodium glycolate.

MODEL PARAMETERS

Parameter	Rat	Estimation Method[a]
Body weight (kg)	0.23	Fixed
Surface area (cm^2)	267	Fixed[b]
Percentage of body weight:		
Liver	2.53	Fixed[c]
Kidney	0.71	Fixed[c]
Lung	1.17	Fixed[c]
Skin	10.0	Fixed[c]
GI tract	3.4	Fixed[c]
Fat	7.0	Fixed[c]
Rapidly perfused	5.1	Fixed[c]
Slowly perfused	91 - Σ(Other tissues)	Fixed[c]
Flows (liters/hr/kg):		
Alveolar ventilation	15.0	Fixed[c]
Cardiac output	15.0	Fixed[c]
Percentage of cardiac output:		
Liver + GI tract	25.0	Fixed[c]
GI tract	21.0	Fixed[c]
Kidney	25.0	Fixed[c]
Skin	5.0	Fixed[c]
Fat	5.0	Fixed[c]
Rapidly perfused	100 - Σ(Other tissues)	Fixed[c]
Slowly perfused	17.0	Fixed[c]

Partition coefficients:

	EG	GA	
Blood:air	17,902	na	Measured
Liver:blood	0.96	0.97	Measured
Kidney:blood	1.22	1.40	Measured
Lung:blood	0.96	na	Measured
Skin:blood	1.19	0.75	Measured
GI tract:blood	1.48	0.95	Measured
Fat:blood	0.64	1.09	Measured
Rapidly perfused:blood	0.96	0.97	Fixed[d]
Slowly perfused:blood	0.57	0.70	Fixed[d]

Parameter	Rat	Estimation Method[a]
Metabolic constants		
EG to GA		
KFEG (hr^{-1})	3.0	Fitted[e]
GA to others		
Km (mg/L)	22.8	Measured[f]
VmaxC (mg/hr/kg)	9.1	Measured[f]
Competitive inhibition of GA by EG		
KI (mg/L)	22.8	Fixed[g]
Urinary clearance		
EG		
KEXEG (L/hr)	0.05	Fitted[e]
GA (see Kidney Model Parameters)		
Absorption		
Oral gavage, EG & GA		
KaO (hr^{-1})	1.0	Fixed[c]
Subcutaneous injection, NaG		
KaSC (hr^{-1})	1.0	Fixed
Intraperitoneal injection, EG		
KaIP (hr^{-1})	1.0	Fixed

[a]Model parameters were either estimated independently and held fixed (Fixed), measured in independent experiments described in Materials and Methods (Measured), or estimated by fitting the model to the data (Fitted).

[b]McDougal et al. (1990).

[c]Corley et al. (1994).

[d]Partition coefficients for rapidly and slowly perfused tissues were set equal to measured values for liver and muscle, respectivley.

[e]First-order metabolism of EG and first-order elimination of EG in urine simultaneously fitted to kinetics of EG in blood and urine from non-pregnant SD rats dosed at 10 and 2500 mg/kg (Pottenger et al., 1998).

[f]Booth and Watson (1999b).

[g]Competitive inhibition constant for GA metabolism (substrate) by EG (inhibitor) arbitrarily set as equal to Km for GA metabolism according to the formula of Tardiff et al. (1997).

KIDNEY MODEL PARAMETERS

Parameter	Female SD	Male SD	Male Wistar
Kidney Weight (% BW, kg)[a]	0.73	0.65	0.62
GFR (l/hr/kg kidney) [a]	41.0	62.1	58.3
Urine Flow (l/hr/kg kidney) [a]	107.5	158.9	66.1
Volume Tubule Urine [b]	0.01*VK	0.01*VK	0.01*VK
Saturable Renal Tubule Reabsorption of GA			
Kt (mg/l)	1.5 [c]	1.5 [c]	17 [d]
TmaxC (mg/hr/kg)	15 [c]	15 [c]	200 [d]

[a] Renal Physiology from Powers (1995).

[b] Volume of urine in proximal tubules arbitrarily set at 1% of kidney volume (used only to calculate urinary elimination and saturable reabsorption based on concentration of GA in tubule urine for in renal model).

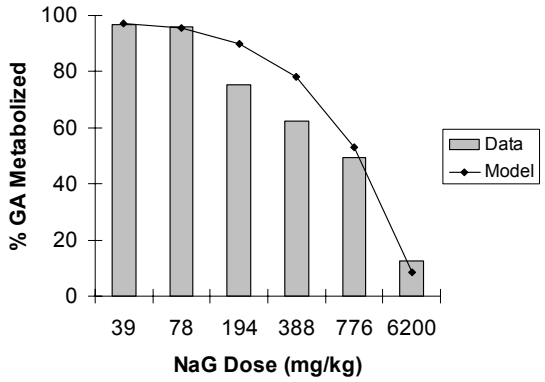
[c] Saturable renal tubule reabsorption of GA estimated from non-pregnant female SD rats administered 10 and 2500 mg/kg EG (Pottenger et al., 1998).

[d] Saturable renal tubule reabsorption of GA estimated from male Wistar rats administered 39, 78, 194, 390, 775 and 6206 mg/kg NaG orally (Harris & Richardson, 1980; Richardson, 1973).

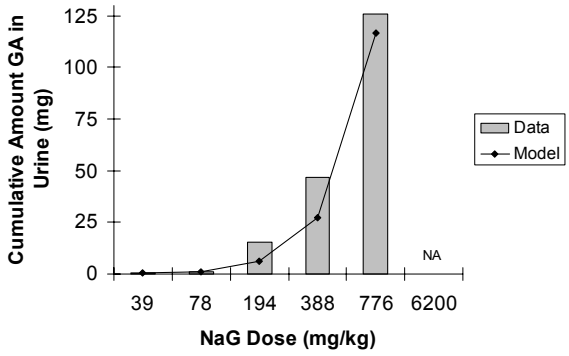
MALE WISTAR RAT – ORAL GAVAGE - NaG

(Richardson, 1973; Harris & Richardson, 1980)

% GA Metabolized



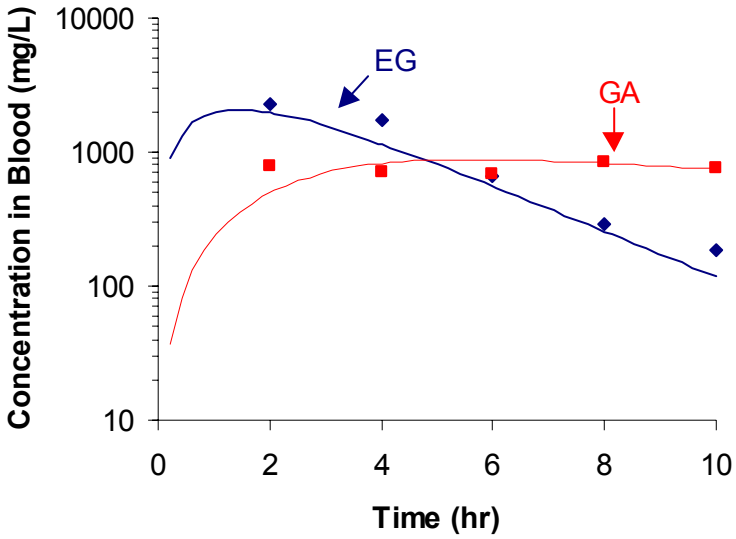
GA Eliminated in Urine



DESIGN: Male Wistar rats dosed orally with NaG at at 39, 78, 194, 388 and 776 mg/kg (Harris & Richardson, 1980) and 6200 mg/kg (Richardson, 1973).

- Cumulative amount of GA in urine 48 hr after oral gavage of NaG used to estimate renal tubule re-absorption of GA in Wistar rats.
- Total amounts of GA metabolized described using measured metabolic rate constants measured in female SD rats, once urinary clearance established.

MALE WISTAR RAT – IP INJECTION - EG
(Chou & Richardson, 1978)



DESIGN: Male Wistar rats injected with [^{14}C]EG at 2700 mg/kg by IP injection.

- Model described the kinetics of EG and GA in blood.

ALBINO RAT – IV INJECTION - EG

(McChesney et al., 1971)

EG in tissues 1 hr after dosing

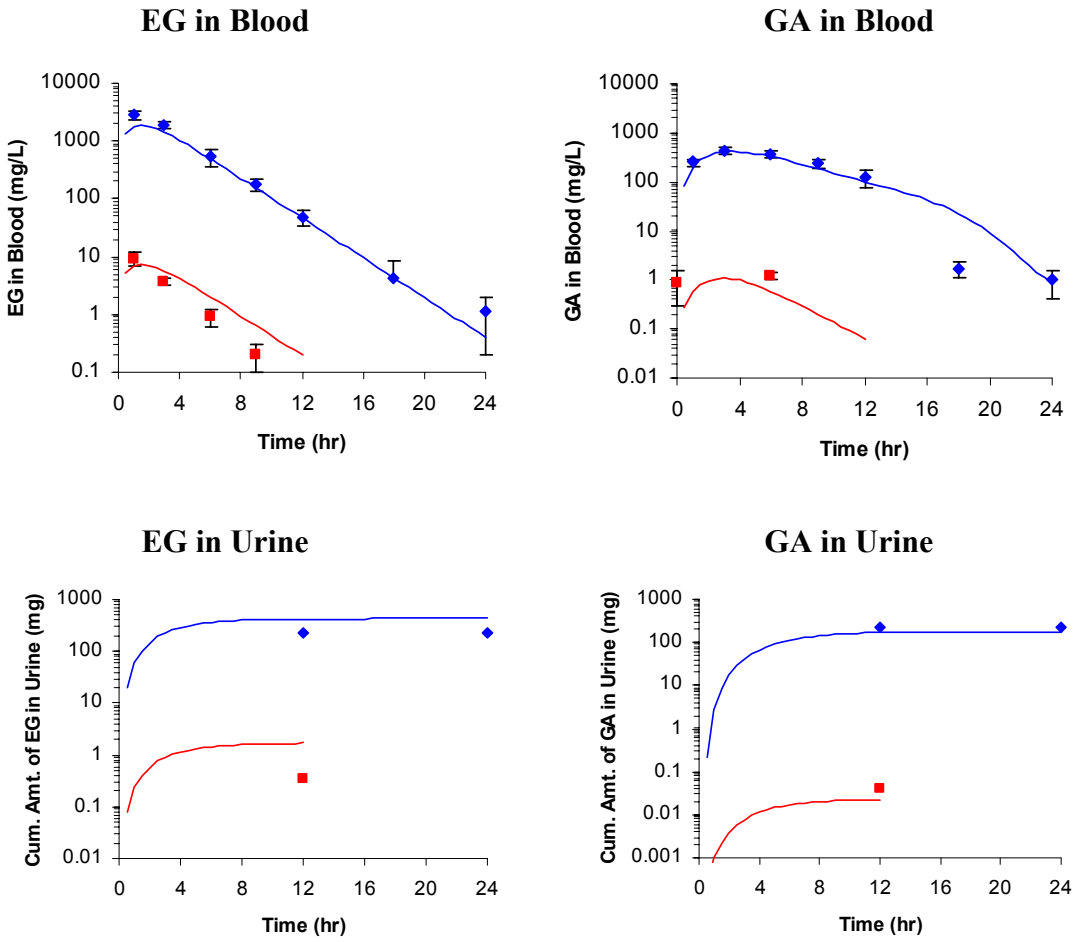
Sample	Observed EG (mg)	Simulated EG (mg)	Ratio (Sim/Obs)
Blood	1.77	1.62	0.91
Lungs	0.28	0.31	1.14
Liver	2.66	0.67	0.25
Kidney	0.18	0.22	1.19

DESIGN: Male albino rats administered [^{14}C]EG at 139 mg/kg by IV injection.

- Model accurately simulated the total amount of EG in blood, lungs and kidneys but significantly underpredicted the amount in the liver 1 hr after dosing.

FEMALE SD RAT – ORAL GAVAGE - EG

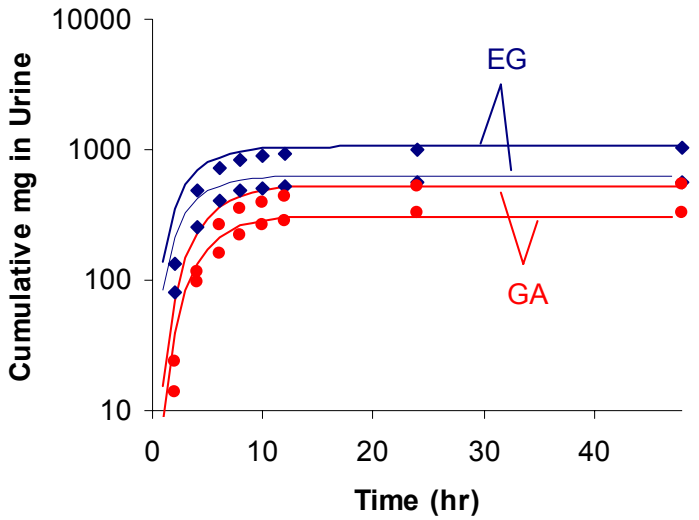
(Pottenger et al., 1998)



DESIGN: Female SD rats were administered EG by gavage at dose levels of 10 and 2500 mg/kg.

- Kinetics of EG in blood and urine used to estimate first-order metabolism of EG to GA and clearance of EG into urine.
- Kinetics of GA in urine and blood used to estimate renal tubule reabsorption of GA.

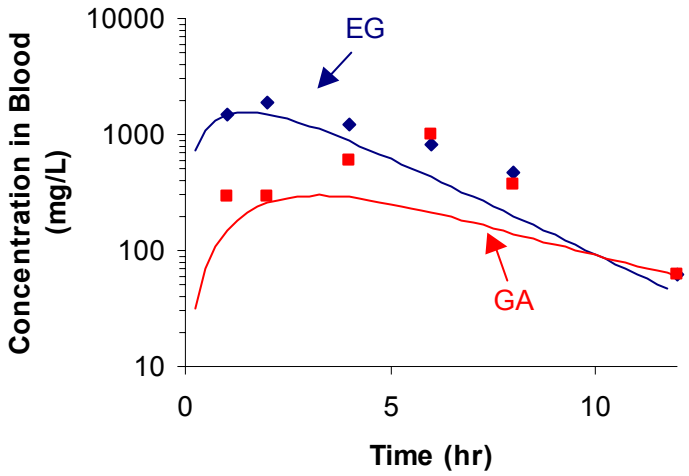
MALE SD RAT – ORAL GAVAGE - EG
(Lenk et al., 1989)



- Model accurately described the kinetics of EG and GA in urine following high oral doses of 3326 and 5544 mg/kg in male SD rats using male SD rat physiology coupled with partition coefficients, metabolism and renal clearance parameters determined from female SD rats.

MALE SD RAT – ORAL GAVAGE - EG

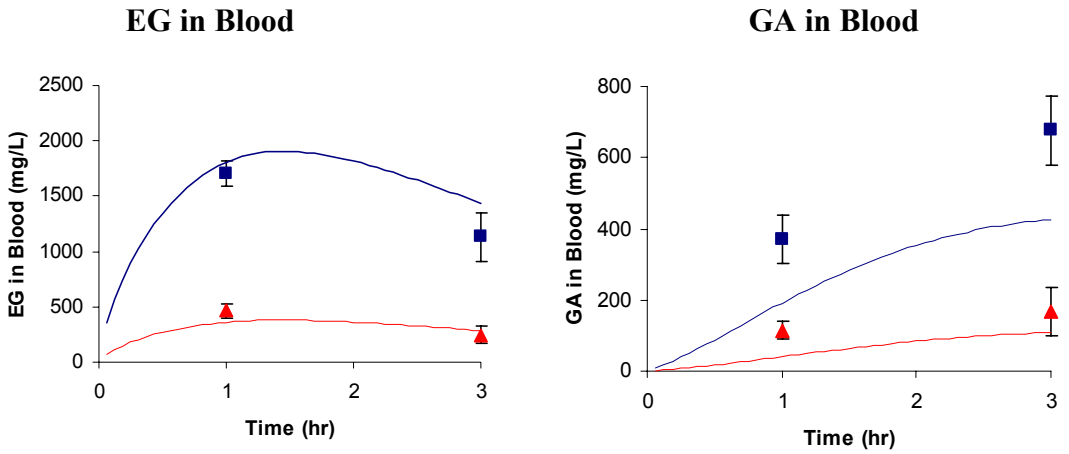
(Hewlett et al., 1989)



- Model described the kinetics of EG in blood following a high dose of 2000 mg/kg in male SD rats.
- GA data appear 'somewhat anomalous' (see 4-8 hr) compared with other data sets and were not as well-simulated by the model.

PREGNANT SD RAT – ORAL GAVAGE - EG

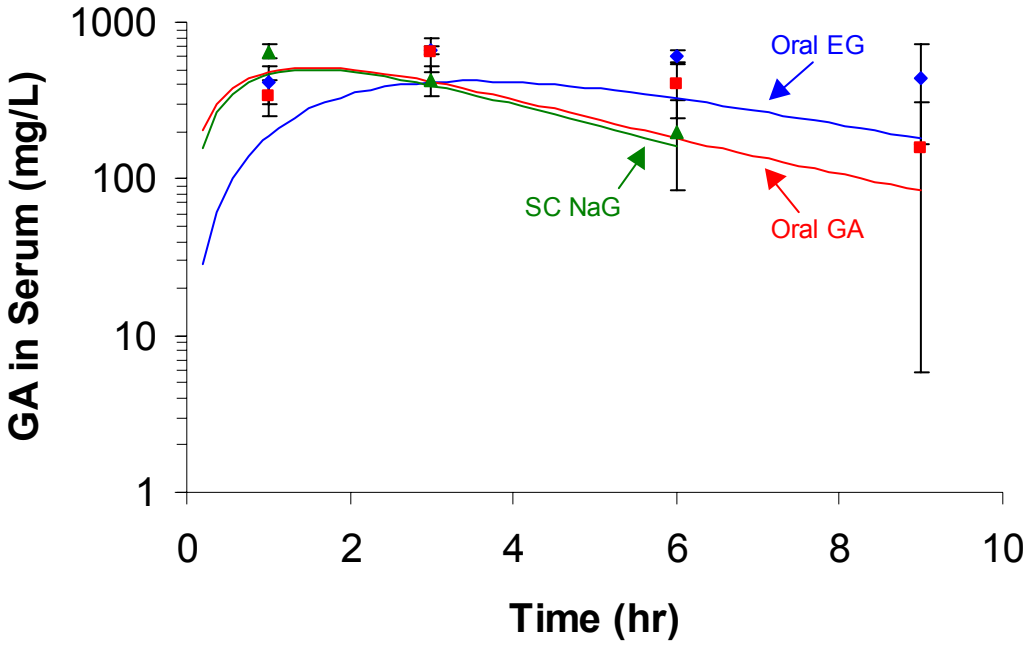
(Carney et al., 1997)



DESIGN: Pregnant SD rats (gd10) were administered EG by gavage at 500 and 1000 mg/kg in a probe study to assist in the design and validation of analytical methods for full kinetic study of Pottenger et al. (1998).

- Model accurately simulated the kinetics of EG in blood.
- Model under-predicted the kinetics of GA at these early time periods.

PREGNANT SD RAT – ORAL GAVAGE – EG & GA
SC INJECTION - NaG
(Carney et al., 1997)



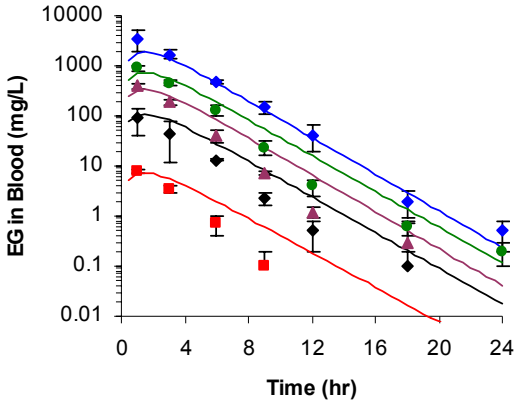
DESIGN: Pregnant (gd10) SD rats administered 2500 mg EG/kg by oral gavage, 650 mg GA/kg by oral gavage or 833 mg NaG/kg by subcutaneous injection (SC) to evaluate the acid-base balance of EG and GA. Dose levels were chosen to attain similar peak concentrations and AUC's for GA in blood for each compound and route of administration.

- Model simulated the more rapid clearance of GA from blood following GA/NaG dosing than observed following EG dosing.
- Data supported the inclusion of competitive metabolism of GA by EG at doses >2000 mg/kg.

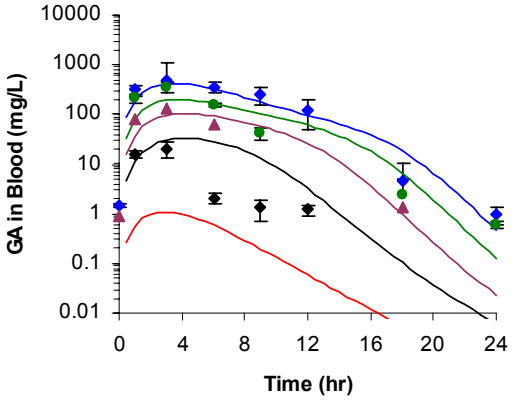
PREGNANT SD RAT – ORAL GAVAGE - EG

(Pottenger et al., 1998)

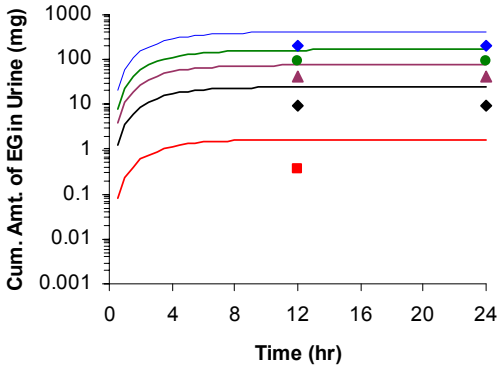
EG in Blood



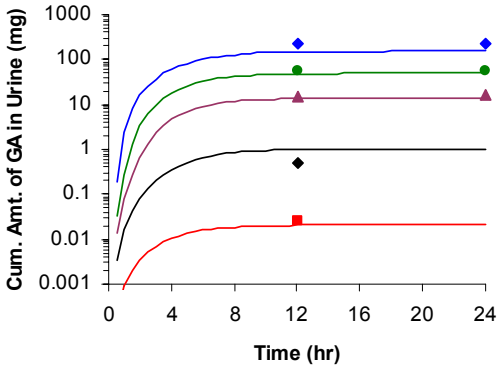
GA in Blood



EG in Urine



GA in Urine

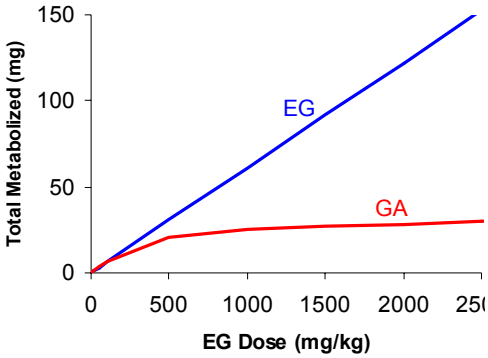


DESIGN: Pregnant SD rats (gd10) were administered EG by gavage at 10, 150, 500, 1000 and 2500 mg/kg.

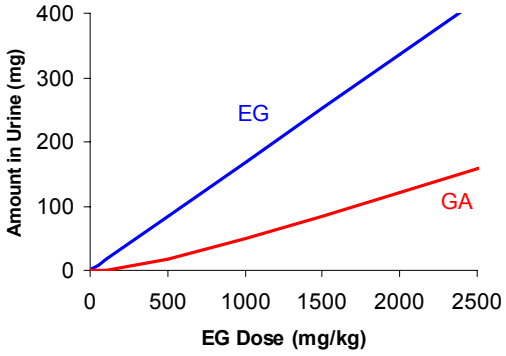
- Model adequately simulated the kinetics of EG in blood but over-predicted elimination into urine using first-order metabolism and renal clearance estimated from non-pregnant female SD rats.
- Model accurately simulated the kinetics of GA in blood and urine using GA metabolism and renal tubule reabsorption estimated from non-pregnant female SD rats.

ORAL GAVAGE DOSE-RESPONSE SIMULATIONS
PREGNANT (GD10) SD RATS

EG/GA Metabolized



EG/GA Excreted in Urine



- Metabolism of GA is saturated ~500 mg/kg while metabolism of EG is linear over dose range simulated.
- Elimination of EG into urine is linear over dose range simulated
- Elimination of GA into urine is non-linear
  - at doses >500 mg/kg EG, GA is eliminated faster than at lower doses

CONCLUSIONS

- Initial PBPK model successfully described the kinetics of EG and its metabolite, GA, in rats following different routes of administration, across a broad range of dose levels and in different strains of male and female rats.
- The non-linear clearance of GA into urine (higher rates observed at doses >2000 mg/kg) required the inclusion of equations describing both glomerular filtration and saturable reabsorption of GA from renal tubules.
- The developmental toxicity of EG in rats is consistent with the non-linear kinetics of GA in maternal blood.
- Ongoing research projects include the kinetics of EG and GA in the embryos, partition coefficients in extraembryonic fluid and embryos, human metabolism of EG and GA, and kidney dosimetry of EG and its metabolites.
- Future efforts will extend the model to humans and to the developing rat embryo.

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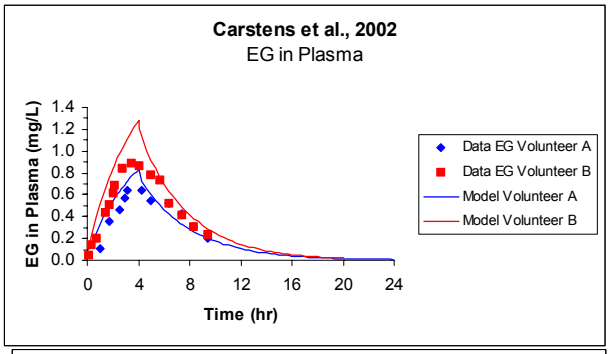
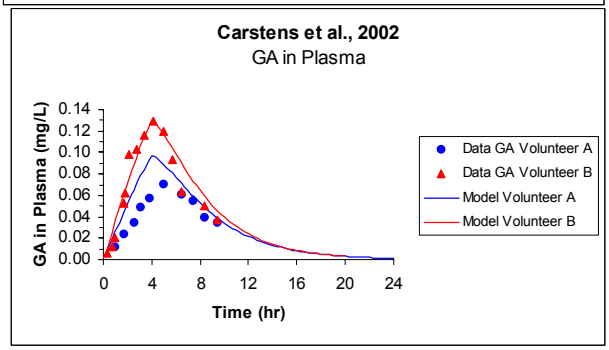
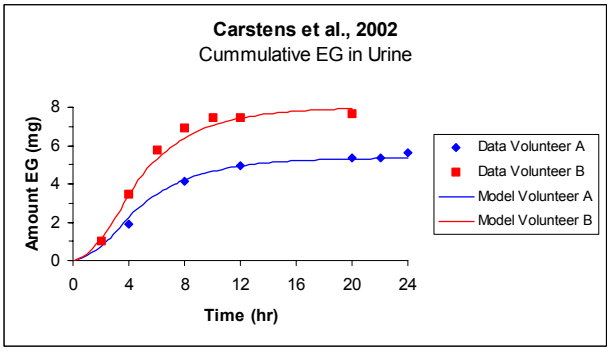
ATTACHMENT #2
EXTENSION OF PBPK MODEL TO THE HUMAN AND SIMULATIONS OF POISONING CASE REPORTS

- **Design**
  - Two volunteers inhaled ^{13}C-EG from heated flask (small amounts injected every 15 min for 4 hr)
  - Blood & urine analyzed for EG, GA and OX
  - No EG detected in exhaled breath
- **PBPK Modeling**
  - Calculated TWA from amount inhaled
  - Metabolism of EG adjusted from data (constant in rats not scaled to BW)
  - Metabolism of GA from Bartels (2001) but optimized Km from data
  - Adjusted renal clearance of EG/GA from data (original rat parameters were not scalable by BW)
  - Renal (and other) physiology from human (ICRP, 1975)







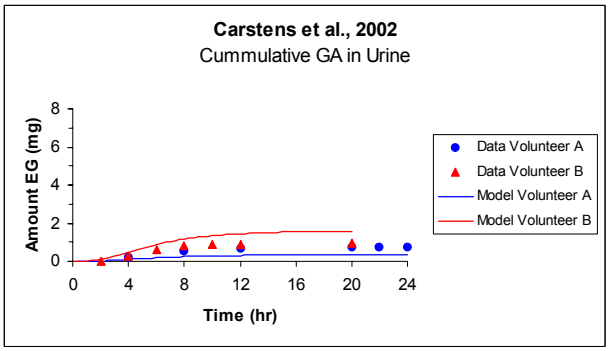


Figure 1. Modifications of rat PBPK model for human simulations using data from Carstens et al. (2002) and human physiology from ICRP (1975).

- Author self-dosed with EG in water at total doses of 5.5, 11 and 13.2 g
- Analyzed EG and OX in urine
- Increased renal clearance of EG 5.6-fold over value that fit Filser data (moderate doses of EG causes diuresis)

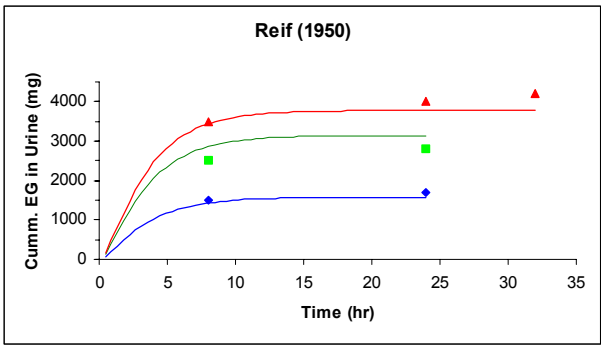


Figure 2. Simulations of human data from Reif (1950).

Figures 3-12 are simulations of human suicide attempt/poisoning case reports. Only the following changes made to the PBPK model to simulate the data:

- Used PBPK model as modified from Filser 2002 data
- Simulations conducted by reducing metabolism of EG to simulate metabolic inhibition by ethanol or 4-MP
- Often body weights, amounts consumed, time since ingestion, or amounts vomited not reported or could not be determined by the hospitals; in these cases:
  - Body weights assumed based upon ICRP Reference Man
  - Dosage adjusted to fit peak blood level (usually 1st blood taken at admission to hospital)

- Male, 30 yr, 74 kg
- Ingested ~100 g EG
- Treatment: 4-MP, gastric lavage, charcoal
- Model adjusted EG metabolism in response to 4-MP inhibition

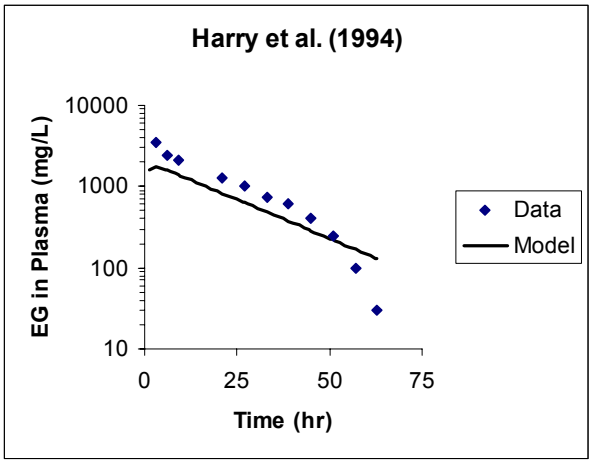


Figure 3. Simulation of human case report of Harry et al., (1994).

- Male, 39 yr, assume 70 kg
- Ingested 500-2000 ml antifreeze
- Treatment: gastric lavage, charcoal, ethanol, bicarb, dialysis

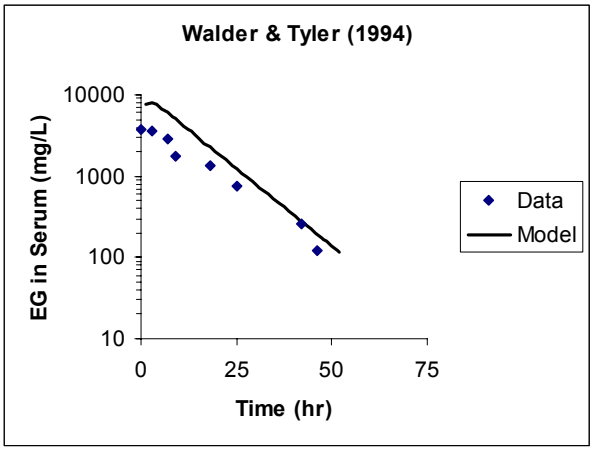


Figure 4. Simulations of human case report of Walder and Tyler, (1994).

- Case 1
  - Male 37 yr, assume 70 kg
  - Ingested “bottles of liquor & antifreeze” then attempted suicide via car exhaust
  - Treatment: ethanol, hemodialysis
- Case 2
  - Male 22 yr, assume 70 kg
  - Ingested unknown quantities of EG and ethanol
  - Treatment: ethanol, bicarb, hemodialysis

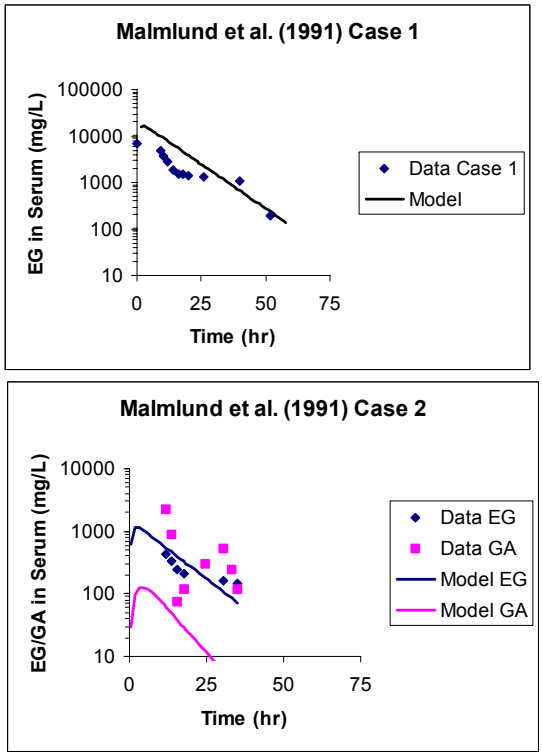


Figure 5. Simulations of human case report of Malmlund et al., (1994).

- Male, 64 yr, assume 70 kg
- Ingested “several cups” of antifreeze
- Treatment: gastric lavage, ethanol, charcoal

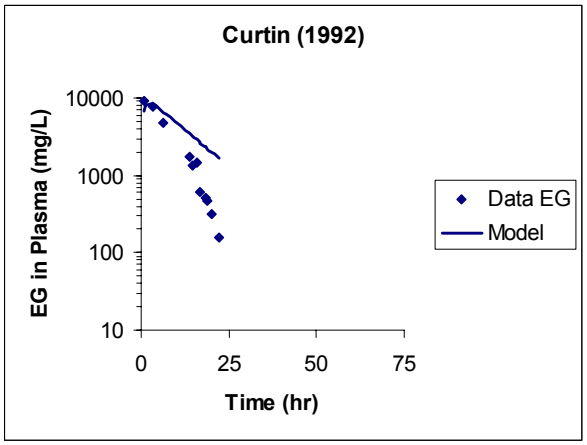


Figure 6. Simulations of human case report of Curtin et al., (1992).

- Case 1
  - Female, 36 yr, 95 kg
  - Ingested “jar” of antifreeze
  - Treatment: naloxone, bicarb, gastric lavage, charcoal, ethanol, hemodialysis
- Case 2
  - Female, 38 yr, 66 kg
  - Ingested ~240 ml antifreeze
  - Treatment: bicarb, ethanol, hemodialysis

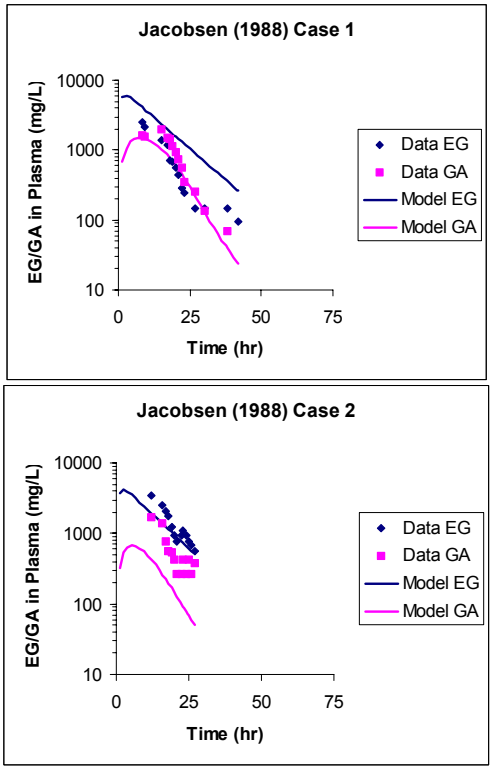


Figure 7. Simulations of human case report of Jacobsen et al., (1988).

- Male, 46 yr, assume 70 kg
- Ingested “glassfull” antifreeze
- Treatment: gastric lavage, charcoal, bicarb, ethanol, peritoneal dialysis

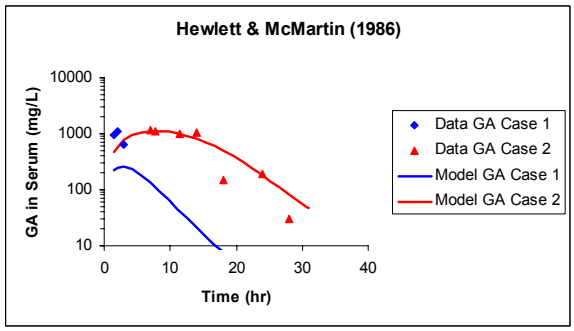


Figure 8. Simulations of human case report of Hewlett and McMartin (1986).

- Case 1
  - Male, 43 yr, assume 70 kg
  - Ingested unknown amount antifreeze
  - Treatment: none reported
- Case 2
  - Male, 42 yr, assume 70 kg
  - Ingested ~500 ml antifreeze and tranquilizers
  - Treatmen: none reported

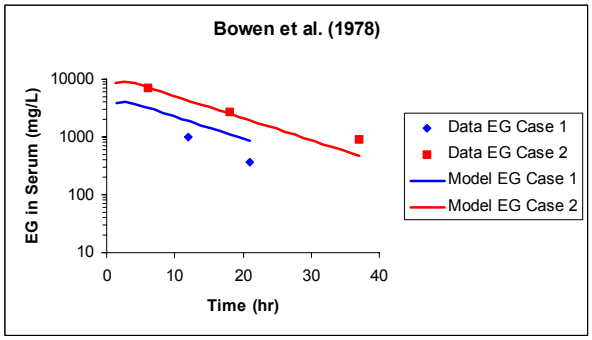
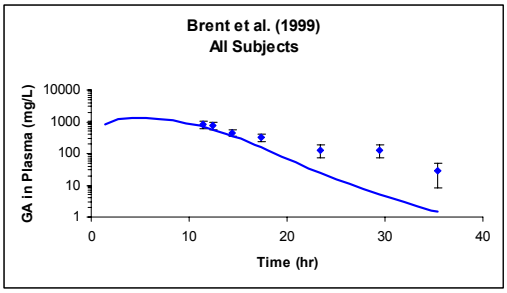
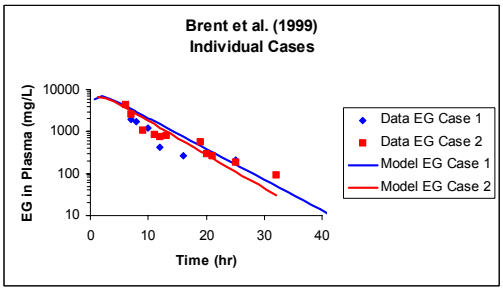


Figure 9. Simulations of human case report of Brown et al. (1978).

- Summary of 19 patients
  - 2 Female/17 Male, avg age 41, assume 70 kg
  - Amounts consumed unknown
  - Treatment: 4-MP, hemodialysis
- Individual Case 1
  - Male, 73 yr, assume 70 kg
  - Ingested unknown amount antifreeze
  - Treatment: 4-MP, hemodialysis
- Individual Case 2
  - Female, 35 yr, assume 60 kg
  - Ingested unknown amount antifreeze
  - Treatment: 4-MP, hemodialysis





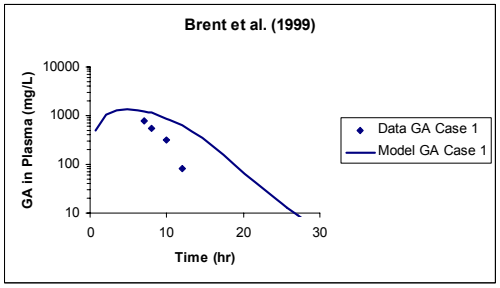
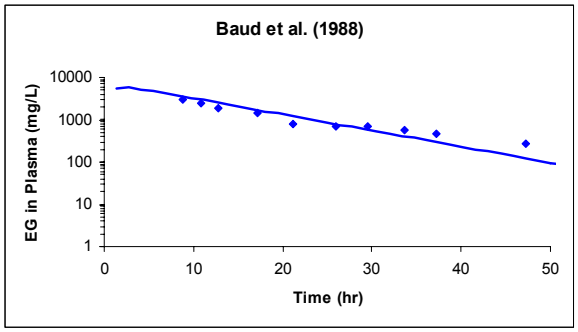


Figure 10. Simulations of human case report of Brent et al. (1999).

- Male, 42 yr, assume 70 kg
- Ingested ~1.5 L antifreeze
- Treatment: gastric lavage, charcoal, bicarb, ethanol, 4-MP



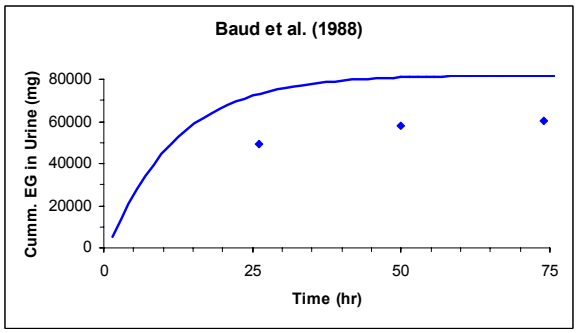


Figure 11. Simulations of human case report of Baud et al. (1988).

- Case 1
  - Male, 19 yr, assume 70 kg
  - Ingested ~300 ml antifreeze
  - Treatment: gastric lavage, bicarb, 4-MP, phenobarb, clonazepam
- Case 2
  - Female, 26 yr, assume 60 kg
  - Ingested 100-200 ml antifreeze
  - Treatment: gastric, lavage, ethanol, bicarb, 4-MP
- Case 3
  - Male, 28 yr, assume 60 kg (Chinese)
  - Ingested ~100 ml antifreeze
  - Treatment: gastric lavage, 4-MP

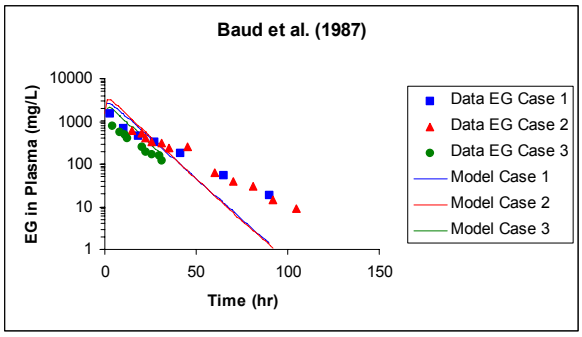


Figure 12. Simulations of human case report of Baud et al. (1987).

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ATTACHMENT #3

Excerpt on species and strain differences in glycolic acid kinetics from: Corley, R.A. (2003). Is the Male Wistar Rat an Appropriate Animal Model for Predicting the Target Tissue Dosimetry of Toxic Metabolites of Ethylene Glycol in Humans? A Comparative Pharmacokinetic Evaluation. R&D Report of Battelle Northwest. Project 29213. Richland, WA.

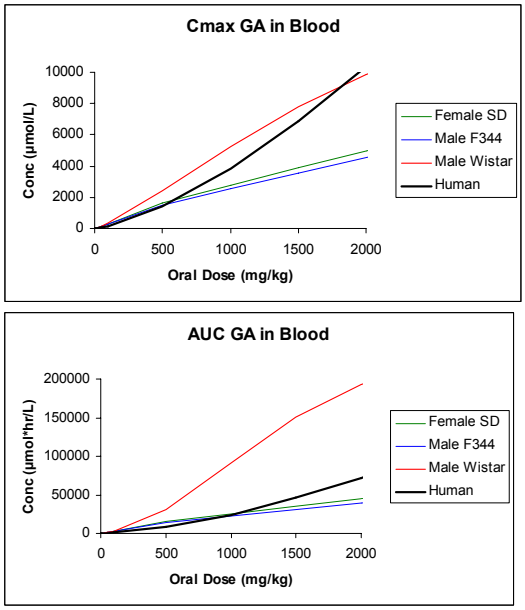


Figure 4. PBPK simulations of the peak (Cmax) and area under the curve (AUC) for glycolic acid in the blood of female SD rats vs. male F344 and Wistar rats and female humans following bolus oral dosing of ethylene glycol. Simulations were conducted assuming 300 g body weight for

Species and Strain Differences in Glycolic Acid Kinetics. The metabolism of ethylene glycol and glycolic acid has been studied in a variety of species and strains of animals. Many of the studies conducted in rats and humans, summarized in **Table 1**, were utilized along with data on strain and species-specific physiology and *in vitro* measurements of tissue solubility, plasma protein binding and metabolism, in the development and validation of a PBPK model for ethylene glycol and glycolic acid (Corley, 2000; Corley et al., 2000). Of particular note is the fact that different strains and sexes of rats have been evaluated. The integration of these data sets into a PBPK model provided a unique format for direct comparisons of blood and tissue levels of the developmentally toxic metabolite, glycolic acid, as a function of dose, dose-rate, and route of exposure.

For example, PBPK simulations of the peak concentrations and areas under the curve for glycolic acid in blood following bolus oral dosing of ethylene glycol (the method of administration used in several developmental toxicity studies) in pregnant Sprague Dawley rats vs. male F344 and Wistar rats and female humans is shown in **Figure 4**. Based upon these simulations, it is apparent that both higher peak concentrations and areas under the curve for glycolic acid in blood are achieved in male Wistar rats vs. other rat strains (and humans) following bolus oral dosing of ethylene glycol.

The rat strain and species differences in the clearance of glycolic acid, as simulated by the PBPK model, are shown in **Figure 5**. Of notable interest is the similarity in the pharmacokinetics of glycolic acid in rat strains other than the Wistar regardless of sex or pregnancy status (Pottenger et al., 2000). Thus, as shown **in Figure 5**, the clearance of glycolic acid in Sprague-Dawley rats, F344 rats and humans can be scaled with an allometric power function

$$Y = aX^b \qquad (1)$$

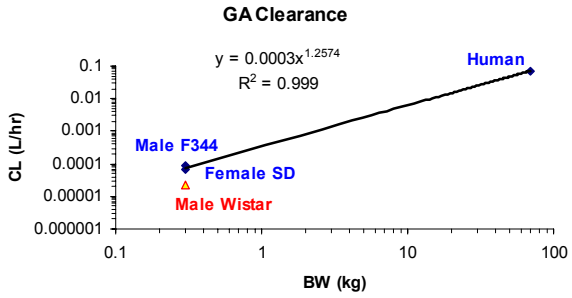
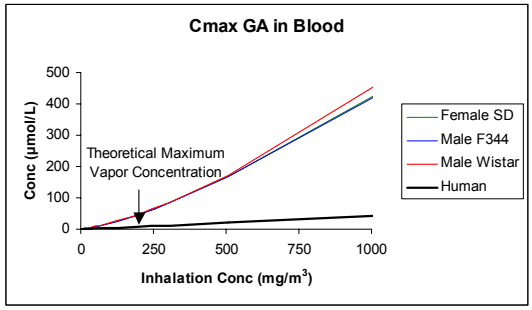


Figure 5. Allometric relationship between body weight and the clearance, defined as dose/AUC, for glycolic acid in female SD rats, male F344 and female humans following bolus oral dosing of 500 mg/kg glycolic acid vs. male Wistar rats. Simulations were conducted assuming 300 g body weight for all rats and 70 kg for human.

where Y is the renal clearance of glycolic acid (L/hr), X is the body weight (kg) and a and b are the coefficient and exponent, respectfully, describing the regression line fit to a log-log plot of the data. In this comparison, the clearance of glycolic acid by male Wistar rats falls below the allometric regression line by a factor of 3.5-fold. Since male Wistar rats achieve larger body weights than male F344 or female Sprague-Dawley rats, these differences will be magnified in older animals.

Similar simulations were conducted for 6-hr, whole-body inhalation exposures to ethylene glycol assuming equal respiratory and dermal bioavailability for vapor and aerosolized ethylene glycol in **Figure 6**. At lower total doses and dose-rates, such as would occur following inhalation exposures to ethylene glycol, the clearance of glycolic acid is not saturated and strain differences are much less significant.



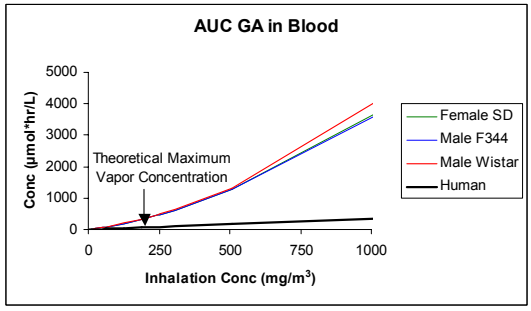


Figure 6. PBPK simulations of the peak (Cmax) and area under the curve (AUC) for glycolic acid in the blood of female SD rats vs. male F344 and Wistar rats and female humans following 6-hr whole-body inhalation exposures to ethylene glycol. Simulations were conducted assuming 300 g body weight for all rats and 70 kg for human.

It can also be seen that humans are not likely to achieve peak blood levels that approach the 2 mM threshold for rat developmental toxicity as suggested by Carney et al. (2000) until bolus oral doses of >500 mg/kg are consumed (**Figure 4**), such as in an attempted suicide, and not at all following inhalation exposures (**Figure 6**). Inhalation exposures to ethylene glycol vapor are limited by its very low vapor pressure (0.06 mmHg at 20°C). Thus, the theoretical maximum vapor concentration is only 79 ppm (~200 mg/m^3). Since some applications may result in aerosolization of ethylene glycol (e.g. aircraft de-icing), 6-hr whole-body simulations were conducted up to 1000 mg/m^3, an atmospheric concentration that is not likely to be tolerated by humans. For example, Wills et al. (1974) have shown that aerosol exposures to concentrations of 188 mg/m^3 can be tolerated by human volunteers for only 15 min while higher concentrations are tolerated even less (e.g. 244 mg/m^3 for 2 min, 308 mg/m^3 for 2 breaths). Thus, human inhalation exposures are limited by low volatility and irritancy of ethylene glycol, which further decreases the chances for inhaling significant amounts of ethylene glycol in occupational exposures. To date, the only substantiated

cases of significant human toxicity to ethylene glycol occur via oral consumption of very large, bolus amounts.

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