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

RA Corley, KK Weitz, RA Gies and KD Thrall
Battelle, Pacific Northwest Laboratory, Richland, WA, USA

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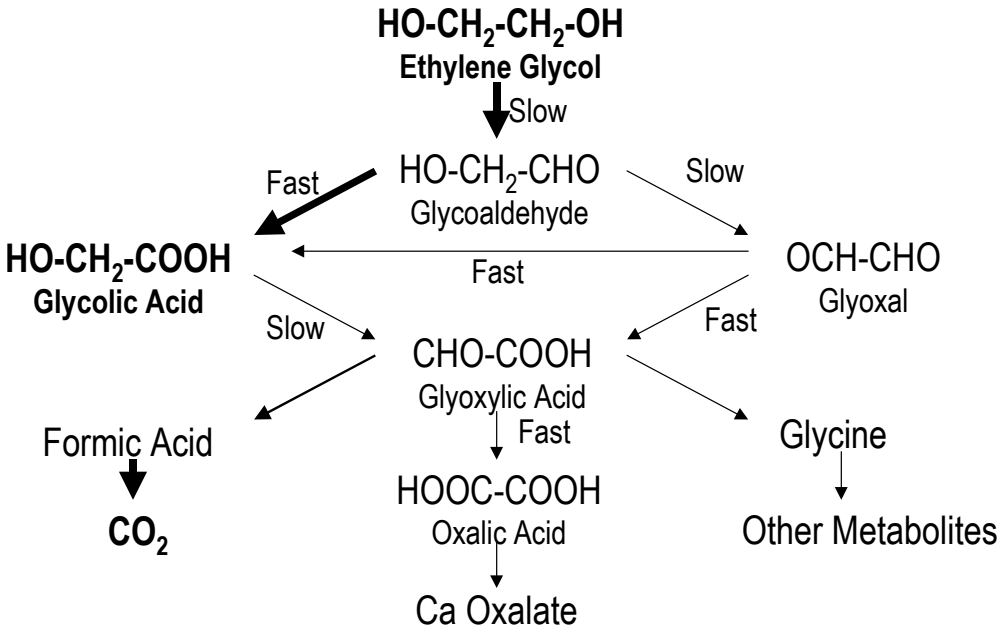
ABSTRACT

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 Chemical Manufacturers Association).

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

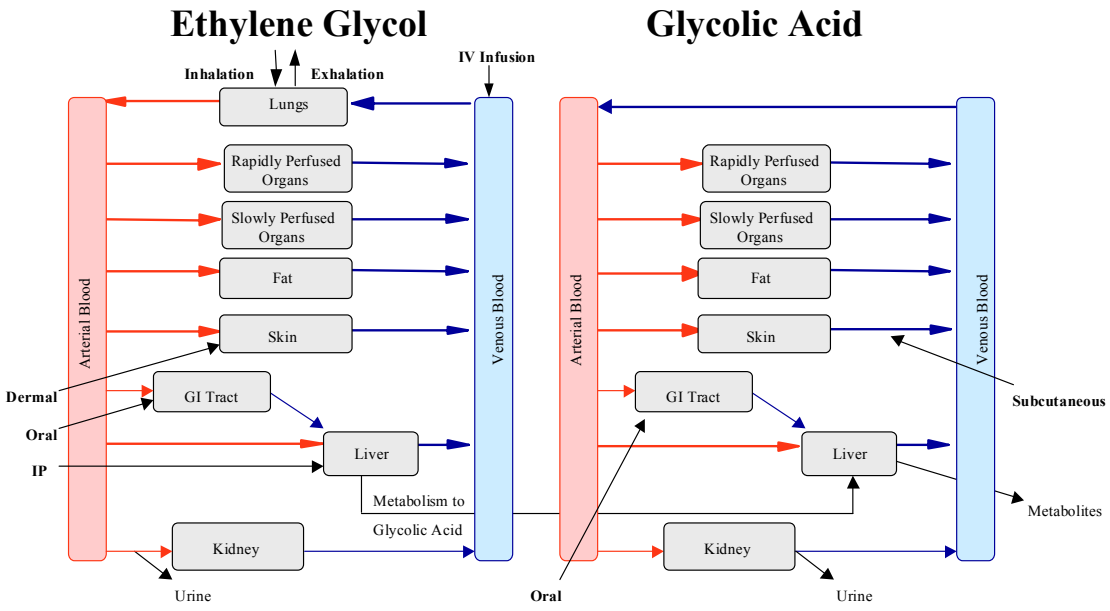
OBJECTIVES

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

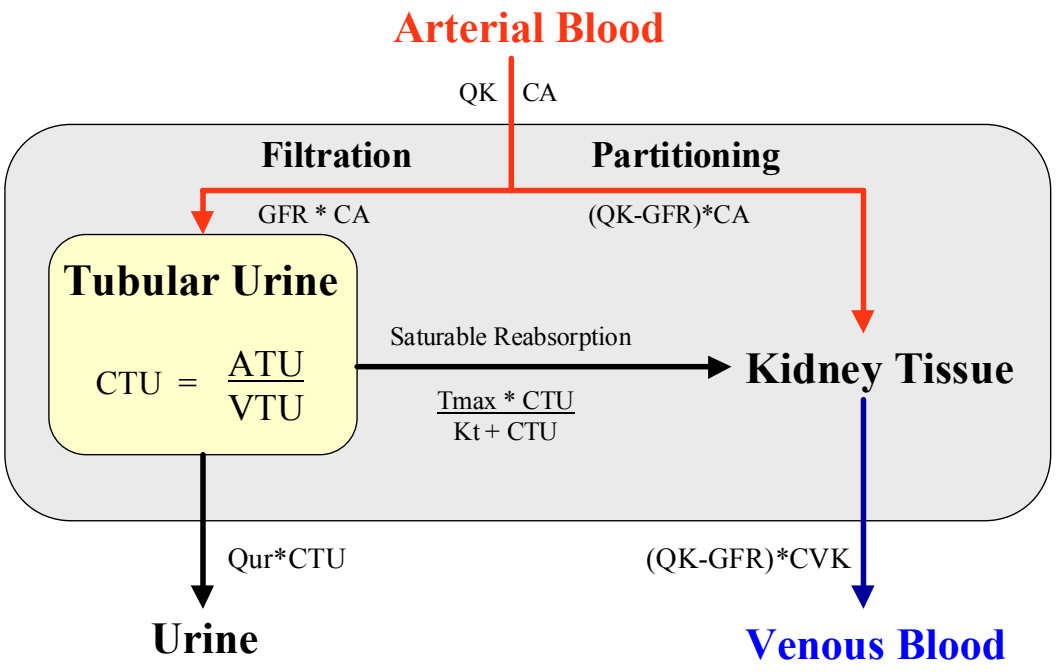
PBPK MODEL STRUCTURE AND ASSUMPTIONS



- **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).

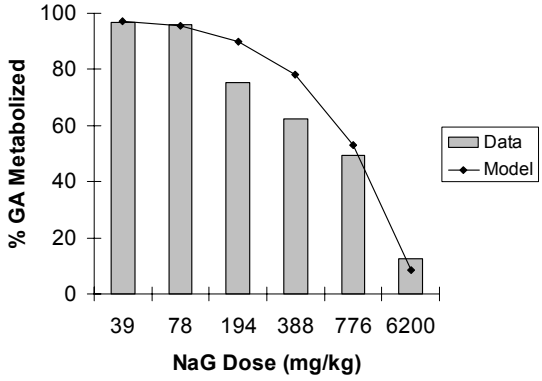
[c] Saturable renal tubule reabsorption of GA estimated from non-pregnant female SD rats administered 10 and 2500 mg/kg EG (Pottenger et al., 1998).

[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).

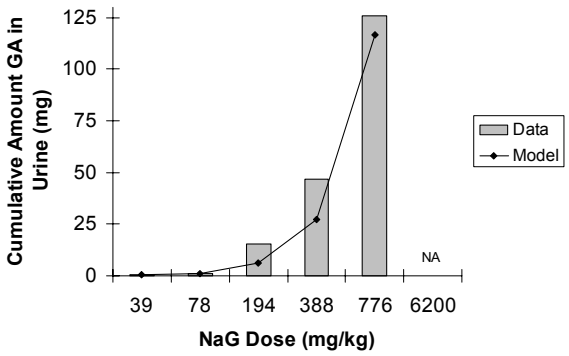
MALE WISTAR RAT – ORAL GAVAGE - NaG

(Richardson, 1973; Harris & Richardson, 1980)

% GA Metabolized



GA Eliminated in Urine

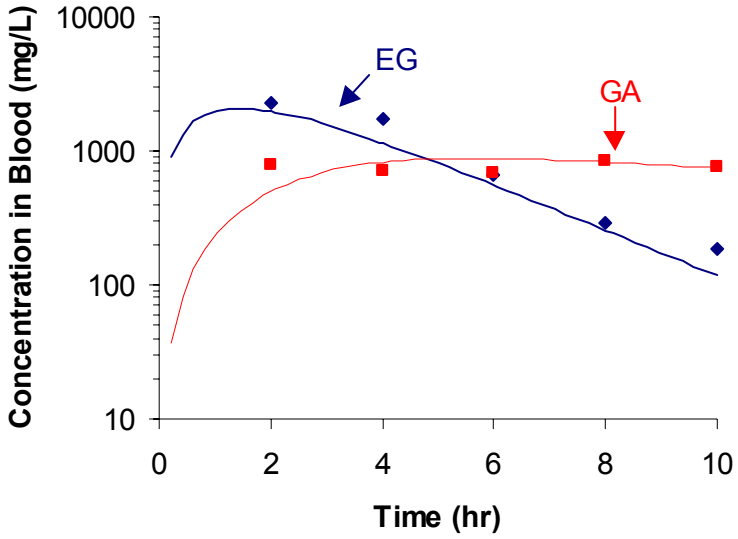


DESIGN: Male Wistar rats dosed orally with NaG at at 39, 78, 194, 388 and 776 mg/kg (Harris & Richardson, 1980) and 6200 mg/kg (Richardson, 1973).

- 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

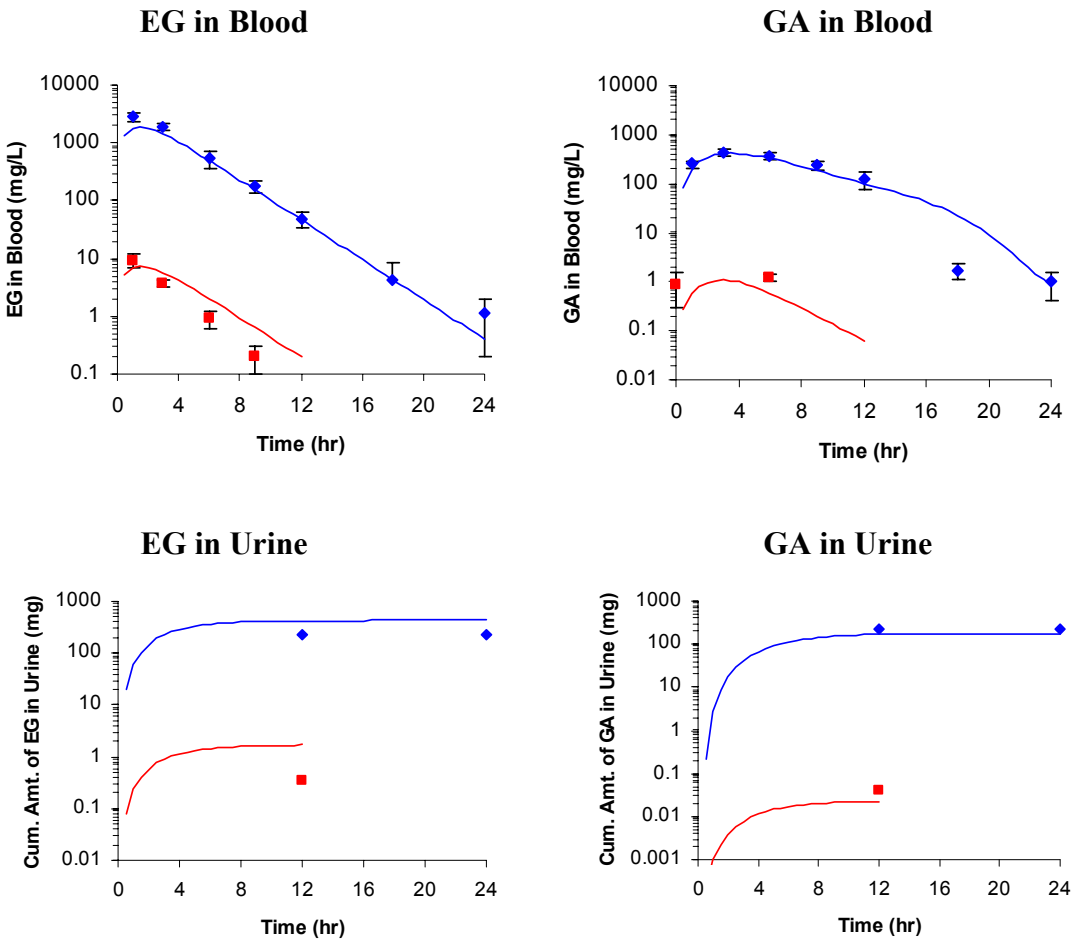
Sample	Observed EG (mg)	Simulated EG (mg)	Ratio (Sim/Obs)
Blood	1.77	1.62	0.91
Lungs	0.28	0.31	1.14
Liver	2.66	0.67	0.25
Kidney	0.18	0.22	1.19

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)

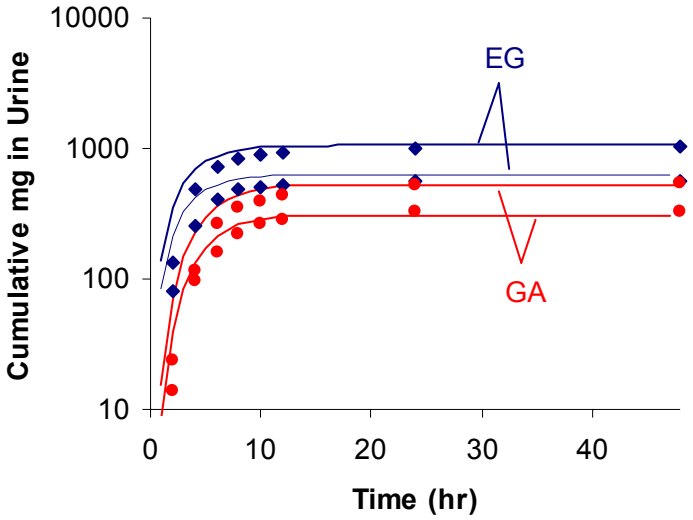


DESIGN: Female SD rats were administered EG by gavage at dose levels of 10 and 2500 mg/kg.

- 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

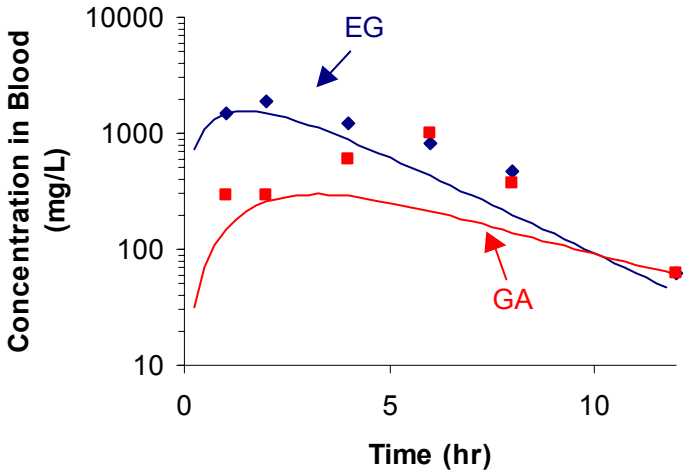
(Lenk et al., 1989)



- 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

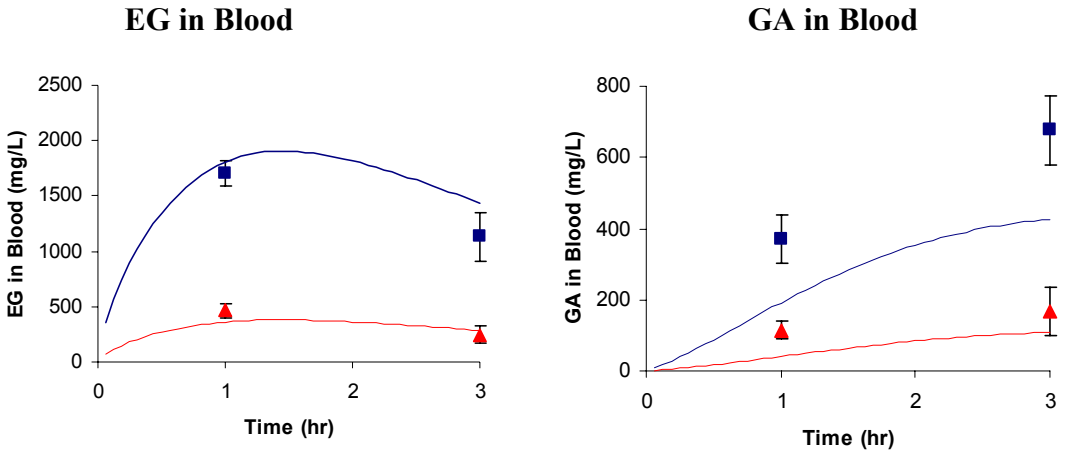
(Hewlett et al., 1989)



- 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

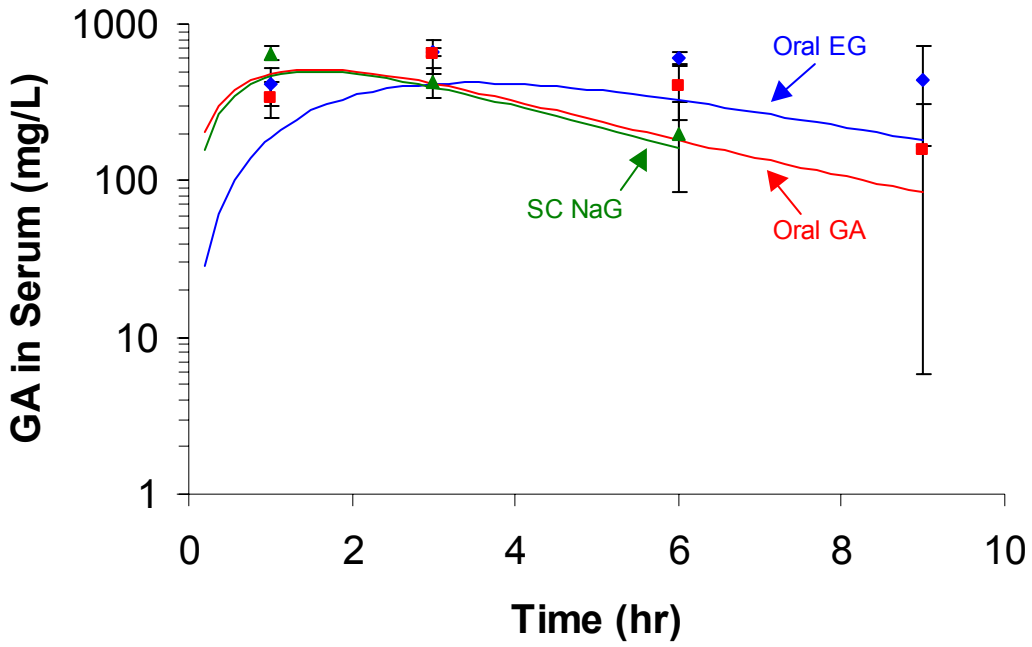
(Carney et al., 1997)



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)



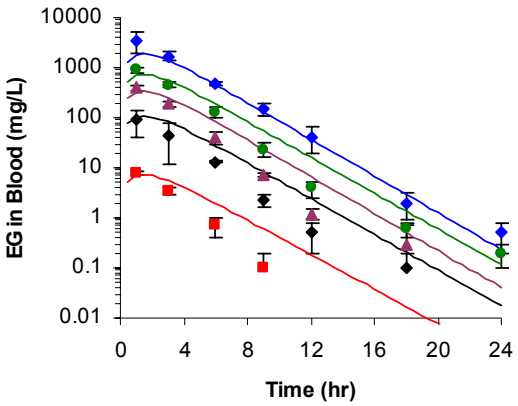
DESIGN: Pregnant (gd10) SD rats administered 2500 mg EG/kg by oral gavage, 650 mg GA/kg by oral gavage or 833 mg NaG/kg by subcutaneous injection (SC) to evaluate the acid-base balance of EG and GA. Dose levels were chosen to attain similar peak concentrations and AUC's for GA in blood for each compound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

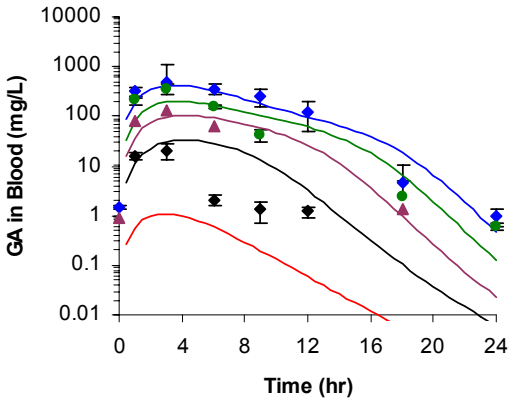
PREGNANT SD RAT – ORAL GAVAGE - EG

(Pottenger et al., 1998)

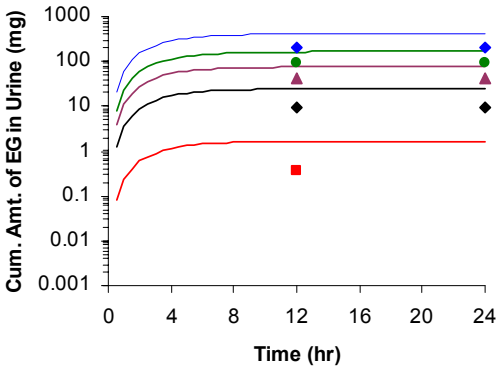
EG in Blood



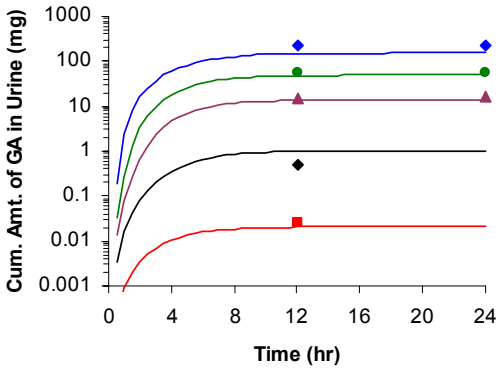
GA in Blood



EG in Urine



GA in Urine

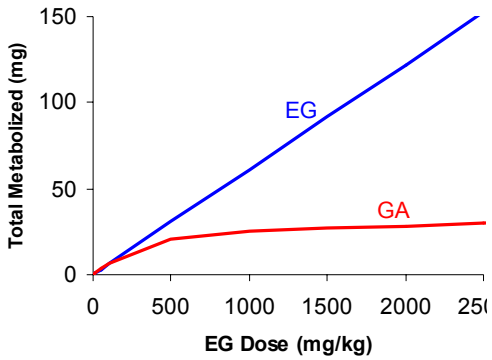


DESIGN: Pregnant SD rats (gd10) were administered EG by gavage at 10, 150, 500, 1000 and 2500 mg/kg.

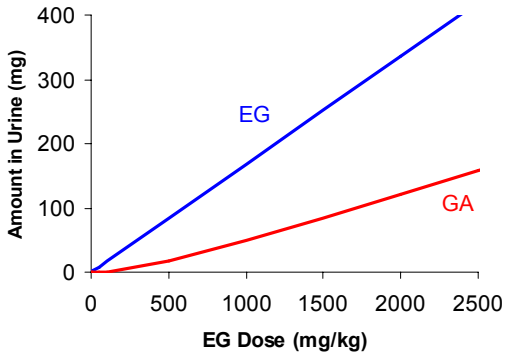
- Model adequately simulated the kinetics of EG in blood but over-predicted elimination into urine using first-order metabolism and renal clearance estimated from non-pregnant female SD rats.
- Model accurately simulated the kinetics of GA in blood and urine using GA metabolism and renal tubule reabsorption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