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ATTN: FYI Coordinator

RE: Union Carbide Corporation's TSCA FYI Submission of June 14, 1984 (et. Seq.) Concerning Ethylene Glycol (CASRN 107-21-1) [FYI-OTS-0684-0323]

Dear Sir or Madam:

As a follow-up to the above-noted submissions concerning ethylene glycol (CASRN 107-21-1), Union Carbide herewith submits the following report.

"Ethylene Glycol Developmental Toxicity Mechanistic Study on the Role of Glycolate Anion and Metabolic Acidosis", Dow Chemical, Lab. Proj. Study ID = K-002558-012A, 9 January 1997 (340pp.).

Please contact the undersigned with questions, if any, at 203/794-5230.

Very truly yours,

William C. Kuryla, Ph.D.
Associate Director
Product Safety

EPA-OTS



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Attachment

Ethylene glycol

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To Tim Cawley
Please file in library
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CHEMICAL MANUFACTURERS ASSOCIATION

January 27, 1997

JAN 31 1997

Give Abstract
to Dr Bellantone

To: Ethylene Glycol Panel
Ethylene Glycol Toxicology Research Task Group

Re: Final Report

TCJ
Bill

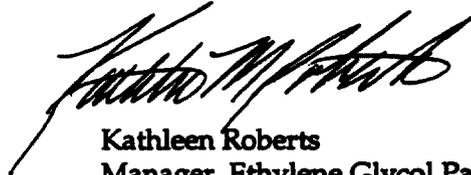
Attached for your files is the following final report:

Ethylene Glycol Developmental Toxicity: Mechanistic Study on the Role of Glycolate Anion and Metabolic Acidosis (January 9, 1997)

This report covers Parts 1 and 2 of the EG Panel's test program on glycolic acid and acidosis. Please note that a copy of this report will be forwarded to Paul Teheux for distribution to the CEFIC MEG Toxicology Work Group.

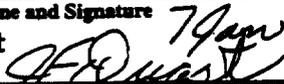
As always, call me with any questions or concerns.

Regards,



Kathleen Roberts
Manager, Ethylene Glycol Panel

DOW CONFIDENTIAL INFORMATION

R & D REPORT HEALTH & ENVIRONMENTAL RESEARCH LABORATORIES THE DOW CHEMICAL COMPANY		CRI Number <hr/> Laboratory Report Code HET K-002558-012
Department THE TOXICOLOGY RESEARCH LABORATORY	Date Issued 9 January 1997	
Title ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS		340 <hr/> PAGES IN FULL REPORT
Author(s) Master Number(s) E. W. Carney/ 246410 N. L. Freshour D. A. Dittenber M. D. Dryzga C. W. Carney 1-9-97 N. L. Freshour 1/8/97 D. A. Dittenber 7 Jan 97 M. D. Dryzga 7 Jan 1997		
Author(s) Signature(s) 		
Reviewer's Name and Signature J. F. Quast 	CAS Number(s) 107-21-1, 79-14-1, 2836-32-0	
DESCRIPTIVE SUMMARY WITH CONCLUSIONS: <p>Previous research suggests that ethylene glycol (EG) developmental toxicity is due to a dose-rate dependent toxicokinetic shift leading to glycolate accumulation and metabolic acidosis. This study was conducted to determine the relative roles of these two factors <i>in vivo</i>. In Part I, carotid artery cannulated pregnant rats received 2500 mg/kg (40.3 mmol/kg) of EG or 650 mg/kg (8.5 mmol/kg) of free glycolic acid (GA) via gavage, or 833 mg/kg (8.5 mmol/kg) of sodium glycolate (NaG; pH 7.4) via subcutaneous (SC) injection. The EG dose was chosen to generate equimolar amounts of glycolate, based on an EG to glycolate conversion rate of 21% (Marshall, 1982). Following exposure, peak serum glycolate levels were nearly identical in all three groups (8.4-8.8 mmol/L). Glycolate area-under-the-curve (AUC) values also were similar for GA and NaG, but were three-fold higher in the EG group. EG and GA caused a clear, but mild, metabolic acidosis, while acid-base status was normal with NaG. In Part II, these three treatments were given on gd 6-15 to groups of 25 time-mated rats, followed by fetal evaluation on gestation day 21. A fourth group of rats was given distilled water via gavage and served as vehicle controls. NaG caused decreased fetal body weights and increased incidences of several minor skeletal variations, effects characteristic of the lowest-observable-effect level (LOEL) observed in previous developmental toxicity studies with EG (Yin et al., 1986; Neepier-Bradley et al., 1995). This result with NaG indicates that the glycolate anion is sufficient to cause developmental toxicity, even in the absence of metabolic acidosis. However, a much greater incidence and severity of fetal effects (including malformations) was observed with GA, indicating that metabolic acidosis is a major exacerbating factor leading to <i>in vivo</i> teratogenesis following large oral bolus exposures to EG. Finally, EG and GA both caused qualitatively similar (often identical) axial skeleton and abdominal wall defects, as well as dilated cerebral ventricles, providing further evidence that glycolate is the proximate developmental toxicant for EG. The only effects which were unique to the EG group were several external malformations involving the cranial neural tube and cranio-facial region. Considering that neural tube and cranio-facial effects were observed in a prior whole embryo culture with GA, but not with EG (Carney et al., 1996), the neural tube and cranio-facial effects found in the this study were likely due to the three-fold higher glycolate AUC value seen in the EG group.</p>		
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Study Title

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Data Requirement

There are no specific data requirements for this study.

Author(s)

E. W. Carney, N. L. Freshour, D. A. Dittenber, and M. D. Dryzga

Study Completion Date

9 January 1997

Sponsor

Ethylene Glycol Panel, Chemical Manufacturers Association
Arlington, VA

Performing Laboratory

The Toxicology Research Laboratory
Health and Environmental Research Laboratories
The Dow Chemical Company
Midland, Michigan 48674

Laboratory Project Study ID

K-002558-012A

COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

Compound: ETHYLENE GLYCOL

Title: ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY:
MECHANISTIC STUDY ON THE ROLE OF GLYCOLATE ANION AND
METABOLIC ACIDOSIS

All phases of this study were conducted in compliance with the Good Laboratory Practice Standards listed below, with the following exceptions:

Test material characterization (provided by commercial suppliers) was not audited for GLP compliance by The Dow Chemical Company.
Dose solutions were not analyzed in the preliminary (Part I) phase of this study.

Food and Drug Administration, Good Laboratory Practice Regulations for NonClinical Studies, Title 21 CFR, Part 58, Final Rule.

United States Environmental Protection Agency, TSCA Good Laboratory Practice Standards, Title 40 Code of Federal Regulations Part 792, 1 July 1990 Edition

Japan Ministry of International Trade and Industry, Good Laboratory Practice Standards Applied to Industrial Chemicals (MITI)

Organisation for Economic Co-Operation and Development
ISBN 92-64-12367-9, Paris 1982

E. W. Carney 1-9-97
E. W. Carney, Ph.D. (Date)
Study Director

J. T. Young 1-7-97
J. T. Young, D.V.M., M.S. (Date)
Director
The Toxicology Research Laboratory

Sponsored and Submitted By:

K. Roberts 12/23/96
K. Roberts (Date)
Chemical Manufacturers Association

QUALITY ASSURANCE STATEMENT

Compound: ETHYLENE GLYCOL

Title: ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY:
MECHANISTIC STUDY ON THE ROLE OF GLYCOLATE ANION AND
METABOLIC ACIDOSIS

This study was examined for conformance with Good Laboratory Practices as published by the U.S. Environmental Protection Agency. The final report was determined to be an accurate reflection of the data obtained. The dates of Quality Assurance activities on this study are listed below.

Study Initiation Date: 2 October, 1995

DATE FINDINGS REPORTED TO STUDY DIRECTOR/MANAGEMENT:

TYPE OF AUDIT:	DATE OF AUDIT:	
Preliminary protocol	27 September, 1995	27 September, 1995
Final protocol	12 October, 1995	12 October, 1995
Protocol, study conduct	12 October, 1995	12 October, 1995
Addendum #1	17 October, 1995	17 October, 1995
Addendum #2	14 November, 1995	14 November, 1995
Addendum #3	17 November, 1995	17 November, 1995
Study conduct	6 March, 1996	8 March, 1996
Addenda #4-6	10 September, 1996	10 September, 1996
Addenda #7-8	20 November, 1996	20 November, 1996
Data and Draft report		
	10 September to 30 October, 1996	31 October, 1996

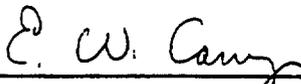
 23 Dec. 1996
K. T. Haut, B.S. (Date)

Quality Assurance
Health and Environmental Research Laboratories
The Dow Chemical Company
1803 Building
Midland, Michigan 48674

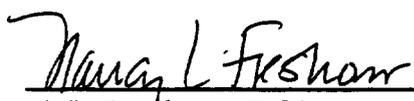
SIGNATURE PAGE

Compound: ETHYLENE GLYCOL

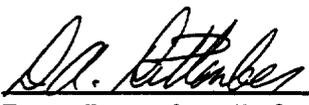
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MECHANISTIC STUDY ON THE ROLE OF GLYCOLATE ANION AND
METABOLIC ACIDOSIS



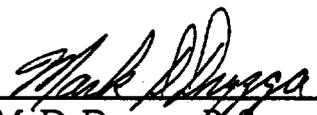
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Study Director (Date)



N. L. Freshour, B.S. 1/8/97
Analytical Chemist (Date)



D. A. Dittenber, B. S. 7/Jan/1997
Clinical Chemistry (Date)



M. D. Dryzga, B. S. 7/Jan/1997
Surgical Specialist (Date)

Reviewed by:



J. F. Quast, D.V.M., Ph.D. 7 Jan 1997
Diplomate, American College of Veterinary Pathologists
Senior Associate Scientist (Date)

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SUMMARY

Previous research suggests that ethylene glycol (EG) developmental toxicity is due to a dose-rate dependent toxicokinetic shift leading to glycolate accumulation and metabolic acidosis. This study was conducted to determine the relative roles of these two factors *in vivo*. In Part I, carotid artery cannulated pregnant rats received 2500 mg/kg (40.3 mmol/kg) of EG or 650 mg/kg (8.5 mmol/kg) of free glycolic acid (GA) via gavage, or 833 mg/kg (8.5 mmol/kg) of sodium glycolate (NaG; pH 7.4) via subcutaneous (SC) injection. The EG dose was chosen to generate equimolar amounts of glycolate, based on an EG to glycolate conversion rate of 21% (Marshall, 1982). Following exposure, peak serum glycolate levels were nearly identical in all three groups (8.4-8.8 mmol/L). Glycolate area-under-the-curve (AUC) values also were similar for GA and NaG, but were three-fold higher in the EG group. EG and GA caused a clear, but mild, metabolic acidosis, while acid-base status was normal with NaG. In Part II, these three treatments were given on gd 6-15 to groups of 25 time-mated rats, followed by fetal evaluation on gestation day 21. A fourth group of rats was given distilled water via gavage and served as vehicle controls. NaG caused decreased fetal body weights and increased incidences of several minor skeletal variations, effects characteristic of the lowest-observable-effect level (LOEL) observed in previous developmental toxicity studies with EG (Yin et al., 1986; Neeper-Bradley et al., 1995). This result with NaG indicates that the glycolate anion is sufficient to cause developmental toxicity, even in the absence of metabolic acidosis. However, a much greater incidence and severity of fetal effects (including malformations) was observed with GA, indicating that metabolic acidosis is a major exacerbating factor leading to *in vivo* teratogenesis following large oral bolus exposures to EG. Finally, EG and GA both caused qualitatively similar (often identical) axial skeleton and abdominal wall defects, as well as dilated cerebral ventricles, providing further evidence that glycolate is the proximate developmental toxicant for EG. The only effects which were unique to the EG group were several external malformations involving the cranial neural tube and cranio-facial region. Considering that neural tube and cranio-facial effects were observed in a prior whole embryo culture with GA, but not with EG (Carney et al., 1996), the neural tube and cranio-facial effects found in this study were likely due to the three-fold higher glycolate AUC value seen in the EG group.

INTRODUCTION

Background. It is well known that large oral doses of EG in rats and mice can lead to systemic and developmental toxicity. In contrast, potential human exposures from typical industrial and consumer uses of EG are more likely to involve the dermal or inhalation routes. In order to address questions regarding the developmental toxicity potential of dermal and inhalation exposures to EG, a working hypothesis (Fig. 1) was developed by the EG Toxicology Research Task Group of the Chemical Manufacturers Association, which integrates the animal developmental toxicity data with the extensive data base describing the absorption, metabolism, pharmacokinetics and mechanisms of systemic toxicity for EG (Carney, 1994).

One of the key tenets of the model is that developmental toxicity is caused by a metabolite of EG, called glycolic acid (GA), and not parent EG. Under most conditions of EG exposure the GA metabolite is present in the blood at very low levels. However, it can become a major metabolite following large doses of EG due to saturation of GA oxidation and/or elimination (Marshall, 1982; Frantz et al., 1996). When levels of this acidic metabolite exceed the capacity of maternal blood buffers to neutralize it, a maternal metabolic acidosis ensues, which has been hypothesized to be the true agent responsible for EG-induced developmental toxicity (Khera, 1991).

The focus on GA and acidosis in the model is based on the following experimental evidence:

- (1) The oral dose of EG at which GA kinetics begin to shift corresponds with the lowest-observable-effect level (LOEL) for developmental toxicity (Marshall, 1982; Neeper-Bradley et al., 1995; Frantz et al., 1996).
- (2) Doses given rapidly are much more effective than similar doses administered over longer time periods, again suggesting that saturation kinetics are important. For example, a gavage dose of 750 mg/kg/day in CD-1 mice resulted in significant developmental toxicity, while a slightly higher dose (800 mg/kg/day) of EG given in drinking water for 14 weeks was a no-effect level.

In rats, 1000 mg/kg/day by gavage resulted in an increased incidence of axial skeleton malformations, but when this same dose was administered through the animals' feed, developmental effects were limited to delayed ossification of vertebral centra (Text Table 1).

- (3) Co-administration of oral sodium bicarbonate with EG almost entirely prevented metabolic acidosis and also partially ameliorated many developmental effects (Khera, 1991).
- (4) In rat whole embryo culture, 12.5 mM GA (at pH 6.7) induced abnormal embryo development, whereas parent EG at up to 50 mM had little effect on embryo development (Carney et al., 1996).
- (5) GA has recently been shown to cause developmental toxicity consistent with EG in rats (Munley, 1996).

TEXT TABLE 1. Summary of development and maternal toxicity studies with EG¹

Species	Developmental		Fetal effects at LOEL
	NOEL	LOEL	
<i>Oral - gavage (mg/kg/day):</i>			
Mouse	n.d.	11,090	Decr. number of viable litters
Mouse	n.d.	750	Decr. body wts, axial skel. malforms.
Mouse	150	500	Extra 14 th rib (variation)
Rat	n.d.	1250	Axial skel. and visceral malforms.
Rat	500	1000	Decr. body wts, axial skel. malforms./vars.
Rat	638	858	Decr. body wts, sternum anomalies
Rabbit	2000	n.d.	None
<i>Oral - feed/drinking water (mg/kg/day):</i>			
Mouse	800	1600	Decr. body wts, facial, skull and rib anomalies
Rat	1000	n.d.	Delayed, unossified vertebral centra
<i>Inhalation (mg/m³):</i>			
Mouse-whole body	150	1000	Decr. body wts, axial skel. And facial malforms.
Mouse-nose only	1000	2500	Decr. body wts, fused ribs, skel. vars.
Rat-whole body	150	1000	Decr. Skel. ossification
<i>Dermal (mg/kg/day):</i>			
Mouse	3549	n.d.	Decr. ossification-parietals, hindlimb phalanges - only effects noted at 3549 mg/kg

¹References cited within Carney (1994).

n.d. = not determined; vars. = variations, malforms.=malformations

The above results clearly indicate that GA kinetics are an important determinant for EG-induced developmental toxicity. However, the role of metabolic acidosis in such toxicity is not as certain. In the rat whole embryo culture study mentioned above (Carney et al., 1996), 12.5 mM sodium glycolate at pH 7.4 caused 58% of the embryos to be malformed, which was similar to the 67% malformation rate for embryos cultured in 12.5 mM GA at acidic pH (pH 6.7). However, only 8% of the embryos cultured in control medium acidified with HCl to pH 6.7 were malformed. Several possibilities are available to explain this *in vitro* result in light

of Khera's (1991) *in vivo* EG/bicarbonate study. First, GA could be the true developmental toxicant, but because it is a weak acid, bicarbonate treatment could have altered its ionization and thus decreased its transfer across the yolk sac/placenta. Alternatively, metabolic acidosis could still be involved through effects on the placenta, as suggested by Khera (1991), or through other physiological parameters which are altered in acidosis (e.g. PCO_2 , HCO_3^- , PO_2 , lactate). In this regard, it is important to note that the placenta is not represented in whole embryo culture, nor does simply lowering medium pH adequately mimic the complex changes which characterize metabolic acidosis *in vivo*.

Objective. This study addressed the question of whether developmental toxicity following high dose exposure to EG *in vivo* was caused by (1) an intrinsic toxicity of the glycolate anion, (2) metabolic acidosis or (3) an additive effect of the two.

Statement of GLP Compliance. Although this is a mechanistic study for which no specific regulatory guidelines exist, the study was conducted in accordance with the Food and Drug Administration Good Laboratory Practice Regulations for NonClinical Studies (Title 21 CFR, Part 58, Final Rule), the Environmental Protection Agency TSCA Good Laboratory Practice Standards (Title 40 CFR, Part 792, Final Rule), the Organisation for Economic Co-Operation and Development Principles for Good Laboratory Practice (European Economic Community, Council Directive 87/18 EEC), the Japan Ministry of International Trade and Industry, Good Laboratory Practice Standards Applied to Industrial Chemicals and the Standard Operating Procedures of The Toxicology Research Laboratory of The Dow Chemical Company.

In addition, in response to the Final Rules amending the U.S. Animal Welfare Act that were promulgated by the U.S. Department of Agriculture effective October 30, 1989, the Animal Care and Use Activity (ACUA) that was required for the conduct of this study were reviewed and given full approval by the Institutional Animal Care and Use Committee (IACUC). The IACUC determined that the proposed Activity was in full accordance with these Final Rules. The IACUC assigned Activity No. Reproductive Toxicology 02, Metabolism 02 and Animal ID 01 to this Animal Care and Use Activity.

MATERIALS AND METHODS

Test Materials:

Ethylene glycol (EG)	Formula: HO-CH ₂ -CH ₂ -OH Formula weight: 62.01 CAS 107-21-1 Source: Aldrich Chemical Company Lot: 07053JZ Purity: 99.98%
Glycolic acid (GA)	Formula: HO-CH ₂ -COOH Formula weight: 76.05 CAS 79-14-1 Source: Aldrich Chemical Company Lot: 06608MZ Purity: 99.7%
Sodium glycolate (NaG)	Formula: HO-CH ₂ -COO ⁻ Na ⁺ Formula weight: 98.03 CAS 2836-32-0 Source: Pfaltz & Bauer, Inc. Lot: S05530 Purity: ≥ 98%

Purity data was limited to that provided by the commercial supplier and was not analyzed further by the testing laboratory.

Test Animals. Time-mated CD® (Sprague-Dawley derived) rats were obtained from Charles River Breeding Laboratories (CRBL; Portage, MI). The CD® rat was selected as a test species based on its general acceptance for developmental toxicity testing, the availability of a reliable commercial source, the availability of historical control data and previous EG developmental toxicity and acid/base studies using this species/strain.

Adult virgin female CD rats, approximately 10 weeks old and weighing approximately 180 - 300 grams (Part I) or 205 - 245 grams (Part II) were naturally mated with one male of the same strain at CRBL. Females were checked for vaginal plugs the following morning and those found with plugs were removed from the males' cage. The day on which a vaginal plug was detected was considered day 0 of gestation.

Upon receipt in the laboratory¹, all animals were examined for health status by a veterinarian. Only healthy animals were used for the study. The animal rooms of the facility were designed to maintain humidity at approximately 40-70%, temperature at approximately 22 °C, photoperiod at 12 hrs light:12 hrs dark and air flow at 10 changes/hour.

Rats used in Part I of the study (preliminary blood glycolate/acid-base study) were randomized on gestation day 10 using a random numbers table, while those in the main study were randomized based on gestation day 0 body weights (provided by CRBL) using a computer-generated procedure designed to increase the probability of uniform body weights across treatment groups. In the preliminary study, the rats were housed in plastic cages provided with ground corn cob bedding material, while rats used for Part II of the study (main study) were housed individually in wire bottom cages. All rats were identified using uniquely coded alphanumeric metal ear tags. Certified Laboratory Rodent Chow No. 5002, Purina Mills, Inc. (St. Louis, MO) and municipal tap water were available *ad libitum*. Analysis of the chow was performed by Purina Mills, Inc. to confirm that the diet provided adequate nutrition and to quantify the levels of selected contaminants associated with the formulation process. Drinking water obtained from the City of Midland was analyzed for chemical constituents and biological contaminants by the City of Midland Water Department. In addition, specific analyses for chemical contaminants were conducted at periodic intervals by an independent laboratory as stated in the Standard Operating Procedures of the Toxicology Research Laboratory, The Dow Chemical Company. The results of the feed and water analyses indicated no contaminants at levels that would interfere with the conduct of this study or interpretation of the results.

Compound Preparation and Administration. EG, GA and NaG were prepared as aqueous solutions in deionized water. GA was prepared as the free acid with no adjustment of pH, while solutions of NaG were titrated to approximately pH 7.4 using HCl. No pH adjustments were made to the EG solutions. In Part I, EG and GA were administered via gavage, such that a dose volume of 10 ml/kg body

¹Fully accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC).

weight yielded the appropriate dose. NaG was administered via subcutaneous (SC) injection using a dose volume of 10 ml/kg body weight. Subcutaneous injections were made in the mid-dorsal region. Part II exposures were conducted as in Part I, except that the dose volume was 4 ml/kg body weight. This reduction in dose volume was done to preclude any problems with repeated SC injections.

The dose solutions used in Part I were not analyzed. However, solutions used in Part II were analyzed prior to the start of dosing to confirm that the targeted concentrations were achieved and at the end of dosing to confirm stability.

Rationale for routes of exposure. Gavage administration was chosen as the route of EG exposure based on previous studies (Table 1) demonstrating the effectiveness of this route for induction of metabolic acidosis and terata. GA given by gavage was also anticipated to cause metabolic acidosis. A pH 7.4 solution of NaG (dose equimolar to GA) was given via subcutaneous injection in order to generate equivalent blood glycolate concentrations, but *without* inducing metabolic acidosis. Through application of the Henderson-Hasselbach equation, one can see that theoretically >99.97% of the glycolate would remain in the ionized form and only <0.03% of the dose would be present as the free acid if NaG were to be administered at a neutral pH site such as the subcutaneous space. Thus, an insignificant amount of acid would be added to the system. Administration of NaG via gavage would not be desirable because the acidic stomach environment would shift the ionization of NaG toward the free acid, potentially increasing the amount of free acid absorbed into the systemic circulation. Calculations regarding the ionization of GA are shown below:

$$\begin{aligned} \text{pH} &= \text{pK}_a + \log \frac{[\text{A}^-]}{[\text{HA}]} && \text{where } \text{pK}_a \text{ of GA} = 3.83 \\ 7.4 &= 3.83 + \log \frac{[\text{A}^-]}{[\text{HA}]} && [\text{A}^-] = \text{glycolate concentration,} \\ 3.57 &= \log \frac{[\text{A}^-]}{[\text{HA}]} && [\text{HA}] = \text{glycolic acid concentration} \\ \underline{3715} &= \frac{[\text{A}^-]}{1} \end{aligned}$$
$$\% \text{ as } \text{A}^- = \frac{[\text{A}^-]}{[\text{A}^-] + [\text{HA}]} \times 100 = \frac{3715}{(3715 + 1)} \times 100 = 99.97\%$$

Experimental Design

1. Part I - Preliminary blood glycolate and acid/base homeostasis study.

Preliminary work was necessary to establish if the dosing strategies described above accomplished the desired objectives and to make any adjustments in dosing regimens if necessary. This was done using cannulated pregnant rats as follows:

Cannulation and dosing. Time-mated rats were implanted with a chronic carotid artery cannula on gestation day 8. The carotid artery was chosen based on Khera's (1991) previous acid-based homeostasis study and the fact that arterial sample is generally preferred for blood pH and blood gas determinations (Brun-Pascaud et al., 1982). Enough rats were prepared to yield at least five rats/group with patent catheters during the majority of sample time points. Under methoxyflurane anesthesia, the right common carotid artery was isolated, clamped and incised with a fine scalpel blade. A cannula containing heparinized saline was then inserted approximately 15 mm into the vessel and ligated in place. The cannula was then tunneled through the subcutaneous space and exteriorized through an incision in the nape of the neck. The distal end of the cannula was fitted with a small length of flexible medical tubing and sealed with a copper wire plug. In the first few rats used in the study, the cannula was composed of polyethylene tubing. Due to problems with blocked cannulae, a switch was made to a softer and more pliable material (Silastic) in an effort to reduce irritancy to the arterial wall and thus improve patency. The rats were allowed to recover for two days following surgery. The cannulae did not interfere with normal mobility of the rats and they were periodically flushed with heparinized saline in an effort to maintain patency.

On gestation day 10, all rats with a patent cannula were randomly allocated into one of four groups until there were a total of five rats/group:

- (1) deionized water via gavage
- (2) 2500 mg/kg (40.3 mmol/kg) of EG via gavage
- (3) 650 mg/kg (8.5 mmol/kg) of GA via gavage

(4) 833 mg/kg (8.5 mmol/kg) of NaG via subcutaneous injection

The EG dose was shown to induce terata in previous studies (reviewed in Carney, 1994), while the GA and NaG doses were based on published pharmacokinetic data in rats indicating that 21% of a 2000 mg/kg I.V. bolus dose of EG is converted to GA (see Marshall, 1982).

Blood sampling. Blood samples (200-300 μ l) were withdrawn from the cannulae into 1 cc syringes at 0 (pre-treatment), 1, 3, 6, 9 and 24 hours post-dosing. These time points were based on the fact that peak plasma glycolate levels and maximum acid/base disturbance were achieved at approximately 4-6 hours post-EG (Hewlett et al., 1989; Khera, 1991; Frantz et al., 1996). The first 200 μ l of blood was transferred into Baxter heparinized blood gas analysis capillary tubes and were used for analysis of acid/base parameters. The remainder of the sample was transferred to a microcentrifuge tube and stored at approximately 4°C to allow the blood to clot. The samples were then centrifuged and the serum was removed and used for serum glycolate analysis.

Acid/base parameters. After each sample collection, the capillary tube was immediately sealed to prevent sample contact with air and was placed on ice until analysis. The sample was transported to the MidMichigan Regional Medical Center (Midland, MI) and analyzed on a Corning Model 278 blood gas analyzer within 90 minutes of collection. The analyzer reports data for pH, PCO₂, PO₂, oxygen saturation, bicarbonate and base excess. Preliminary methods development work verified the stability of the acid-base determinations for up to 90 minutes post-sampling.

Blood glycolate analysis. Blood serum was stored at -80° C for subsequent analysis for total glycolate concentration (i.e., the method quantitated the sum of both ionized and non-ionized forms). Serum samples were thawed to room temperature, then mixed briefly using a vortex mixer. An aliquot (25- μ l) of each serum sample was placed in a vial and 0.5 ml of 0.1 N o-phosphoric acid in acetonitrile (approximately pH 1.5) was added to convert glycolate ions present to the protonated glycolic acid form for analysis. The samples were

extracted with the acidified acetonitrile for 15 minutes using an automated vortex mixer. The samples were then placed in a centrifuge for 5 minutes at 3000 rpm. The acetonitrile supernatant extracts were analyzed for total glycolate concentration (detected as glycolic acid) by reversed-phase high pressure liquid chromatography (HPLC) using ultraviolet detection (UV) at 200 nm. The analytical column employed was a 250 x 4.6 mm YMC ODS-AQ column (YMC, Inc. Wilmington, DE) with a corresponding guard column cartridge. The mobile phase was 0.02 M monobasic sodium phosphate (aq.) adjusted to pH 2.8 with o-phosphoric acid at a flow rate of 0.5 ml/min. Sample injection size was 50 microliters.

External standards, prepared in extraction solvent and bracketing expected study sample concentrations, were used to quantitate the glycolic acid peak in the sample extracts. The mean response factor for the UV response in the linear portion of the response curve was used to calculate results. Fortified serum samples (spikes) were prepared covering the expected concentration range of the study samples. They were analyzed with each sample set to determine recovery of glycolic acid. The recovery was non-linear over the concentration range of the study samples; study sample concentrations were adjusted for spike recovery using the recovery factor derived from the spike that most closely matched sample peak area.

Animal observations, body weights and disposition. Animals were observed daily for alterations in behavior or demeanor. Body weights were taken on gestation day 8, 10 and 11. Feed consumption was not determined. Following collection of the last blood sample on gestation day 11, the rats were sacrificed via CO₂ inhalation, cannula placement was verified, and the uterine implantation sites were examined grossly. Animals found not pregnant were excluded from the analyses. A gross necropsy was performed by study personnel for any animal which died or appeared moribund prior to the scheduled sacrifice.

Data evaluation and interpretation. Means and standard deviations were calculated for serum glycolate concentrations, arterial pH, PCO₂, and bicarbonate. The serum glycolate concentration time curve was then plotted

on a log scale to determine peak concentration (C_{max}) and time to peak concentration (T_{max}). Area under the serum glycolate concentration curve (AUC) was calculated using the trapezoidal rule, and elimination constant (K_{elim}) and elimination half-life ($t_{1/2\ elim}$) estimates were derived from slope analyses as described by Gibaldi and Perrier (1982).

Part I study data were submitted to the Sponsor upon completion. After reviewing these data, approval to proceed with Part II of the study was granted.

2. Part II - Developmental toxicity study

Groups of 25 (non-cannulated), time-mated CD rats were exposed on gestation day 6-15 to one of the following: (1) deionized water via gavage (2) 650 mg/kg/day of GA via gavage (3) 833 mg/kg/day of NaG via SC injection or (4) 2500 mg/kg/day of EG via gavage. These dosing regimens were based on the results of Part I.

All animals were observed daily during the study for alterations in behavior or demeanor. Any animal which died, appeared moribund or showed indications of early termination of pregnancy was submitted for a gross pathological examination conducted by a veterinary pathologist. Body weights were recorded on day 0 (provided by CRBL), 6-16, 19 and 21 (terminal body weight) of gestation. Dose volumes were adjusted daily based on body weights. Feed consumption was determined at 3-4 day intervals beginning on day 3 of gestation.

On day 21 of gestation, all surviving animals were euthanized by carbon dioxide inhalation and given a limited necropsy. Any obvious structural or pathologic changes noted in the adult were recorded and the weights of the liver, kidneys and gravid uteri were recorded. The uterus with attached placentae (fetuses removed), liver, kidneys and any gross lesions were preserved in neutral, phosphate-buffered 10% formalin, but microscopic examination of tissues was not conducted.

Fetal Observations. At necropsy, the uterine horns were exteriorized through an abdominal incision and the following data were recorded: 1) the number and position of fetuses *in utero*, 2) the number of live and dead fetuses, 3) the number and position of resorptions, 4) the number of corpora lutea, 5) the sex and body weight of each fetus, and 6) any gross external alterations. The uteri of animals which appeared non-pregnant were stained with a 10% aqueous solution of sodium sulfide (Kopf et al., 1964) and examined for evidence of early resorptions. Corpora lutea were not counted for females that were not visibly pregnant at Cesarean-section or for females that were submitted for necropsy prior to day 21. At least one-half of the fetuses in each litter, selected using a computer-generated randomization procedure, were immediately examined by dissection under a low power stereomicroscope for evidence of visceral alterations (Staples, 1974). The heads of rat fetuses examined by dissection were removed, placed in Bouin's fixative and examined by the serial sectioning technique of Wilson (1965). All fetuses were then preserved in alcohol, eviscerated and stained with alizarin red-S (Dawson, 1926). Skeletal examinations were conducted on all fetuses that were not given visceral examinations.

Statistical Evaluation. In the methods development study, only descriptive statistics (means and standard deviations) for glycolate and acid/base parameters were calculated. In the main study, maternal body weights, body weight gains, organ weights, reproductive parameters and mean fetal body weights were evaluated by Bartlett's test for equality of variances. Based on the outcome of Bartlett's test, a parametric or nonparametric analysis of variance (ANOVA) was performed. If the parametric or nonparametric ANOVA were significant, analysis by Dunnett's test or the Wilcoxon Rank-Sum test with Bonferroni's correction were performed, respectively. Statistical evaluation of the frequency of pre-implantation loss, resorptions and fetal alterations among litters and the fetal population were performed using a censored Wilcoxon test with Bonferroni's correction. The number of corpora lutea and implants, and litter size were evaluated using a nonparametric ANOVA followed by the Wilcoxon Rank-Sum test with Bonferroni's correction. Pregnancy rates were analyzed using the Fisher exact probability test. Descriptive statistics for feed consumption data were calculated with no further statistical analysis. Nonpregnant females, females pregnant following staining, or females having totally resorbed litters were excluded from the appropriate analyses. Fetal sex ratios were evaluated using a

binomial distribution test. Statistical outliers were identified using a sequential method, but only values for feed consumption were routinely excluded unless justified by sound scientific reasons unrelated to treatment.

The nominal alpha levels were as follows:

Bartlett's Test (Winer, 1971)	$\alpha = 0.01$
Parametric ANOVA (Steel and Torrie, 1960)	$\alpha = 0.10$
Nonparametric ANOVA (Hollander and Wolfe, 1973)	$\alpha = 0.10$
Dunnett's Test (Winer, 1971)	$\alpha = 0.05$, two-sided
Wilcoxon Rank-Sum Test (Hollander and Wolfe, 1973)	$\alpha = 0.05$, two-sided with Bonferroni's correction (Miller, 1966)
Fisher's Test (Siegel, 1956)	$\alpha = 0.05$, two-sided
Censored Wilcoxon Test (Haseman and Hoel, 1974)	$\alpha = 0.05$, two-sided
Sequential Outliers Test (Grubbs, 1969)	$\alpha = 0.02$, two-sided
Binomial Distribution Test (Steel and Torrie, 1960)	$\alpha = 0.05$, two-sided

Because numerous measurements were statistically compared in the same group of animals, the overall false positive rate (Type I errors) was expected to be much greater than the cited alpha levels suggested. Therefore, the final interpretation of the numerical data also took into consideration whether or not the results were significant in light of other biologic and pathologic findings.

Quality Assurance. The conduct and data generated during this study were reviewed according to the procedures of the Quality Assurance Unit of The Dow Chemical Company, Health and Environmental Research Laboratories. Permanent records of all data generated during the course of the study, the protocol, any addenda, and the final report were available for inspection by the Quality Assurance Unit. All data generated, including the protocol, addenda, and final report are archived at Health and Environmental Research Laboratories, The Dow Chemical Company, Midland, Michigan.

RESULTS AND DISCUSSION

Part I

Acid-base balance. As shown in Figure 2 and Table 1, both EG and GA produced a mild metabolic acidosis, with a maximal decrease in blood pH of approximately 0.1 unit and which persisted for at least 9 hours, but was completely resolved by 24 hours post-dosing. The time course and magnitude of these changes were nearly identical in these two groups. These changes also were very similar to those observed by Khera (1991) in rats following an oral dose of 2500 mg/kg/day on gestation day 11. Corresponding with the mild metabolic acidosis in the EG and GA groups was a slight decrease in blood bicarbonate level. PCO₂ also was decreased slightly in the EG group, and to a barely perceptible degree in the GA group. In contrast, treatment with NaG did not cause any alterations in blood pH, PCO₂ or bicarbonate relative to control values.

Blood glycolate analysis. Figure 3 and Table 2 show the serum glycolate concentration data for gestation day 10 rats given EG, GA or NaG. Serum glycolate was expressed in Figure 3 on a log scale so that a slope of the elimination phase of each curve could be calculated. Pharmacokinetic values derived from the curves are summarized in Table 3.

Administration of 2500 mg/kg of EG resulted in serum glycolate levels which generally were in the millimolar range, with a peak concentration of 8.8 mmol/L of glycolate occurring at the 3 hour blood-sampling interval. This value corresponds with the previously mentioned whole embryo culture study (Carney et al., 1996), in which embryo development was affected at ≥ 12.5 mmol of glycolic acid/L, while 2.5 mmol/L was the no-effect-concentration. In male rats gavaged with 2000 mg/kg of EG, peak plasma glycolate was approximately 13 mmol/L (Hewlett et al., 1989). The lower value in pregnant rats relative to male rats may possibly be due to the expansion of blood volume which occurs during pregnancy, thus resulting in hemodilution. In this study with pregnant rats, AUC for serum glycolate was calculated to be 146.6 mmol L⁻¹ h. Extrapolation of the elimination phase of the curve indicated a half-life of approximately 10 hours for

elimination of glycolate from blood. Serum glycolate levels were undetectable by 24 hours after dosing with EG.

Both GA administered by oral gavage and NaG given via SC injection resulted in blood glycolate levels which were in a very similar range as for the 2500 mg/kg dose of EG. In fact, peak glycolate levels in all three groups were within 5% of one another (8.4-8.8 mmol/L). In comparing the GA and NaG groups to one another, it was found that most pharmacokinetic values were also very similar. The major difference between the two curves was the earlier T_{max} of NaG, reflecting the more rapid rate of absorption associated with its SC route of administration. Most likely, blood glycolate levels in the NaG group peaked prior to the first sampling point of 1 hour, which, if determined, would have increased the C_{max} and AUC values slightly. In any case, the two curves appeared remarkably similar, almost overlapping one another if one accounts for the differences in absorption time. There was no detectable glycolate in any of the 24 hour samples from the GA or NaG groups, nor was there any in the 9 h samples in the NaG group.

While peak glycolate levels were very similar in the EG, GA and NaG groups, glycolate tended to persist in the serum for longer amounts of time when EG was administered vs. when GA or NaG was given. This resulted in an $AUC_{glycolate}$ value for the EG group which was three-fold higher than that following GA or NaG exposure. Several factors may be responsible for this phenomenon. One likely explanation is a greater competition for rate-limiting oxidation potential when all of the reactions in the EG pathway are operative (EG group) compared to when the initial reactions are by-passed when administering the glycolate metabolite (GA, NaG groups). EG presumably persists in the blood for a period of time, during which conversion of EG to GA continuously takes place. In contrast, there is no opportunity for continued GA production in the GA group. Finally, renal elimination of glycolate also could differ slightly between the EG and GA/NaG groups.

In-life observations, body weights and gross necropsy. There were no clinical observations which were related to treatment with any of the test compounds (Table 4). Observations apparently related to complications of the arterial catheter occurred in two control rats and one rat in the NaG group. Control group

rat 95A8122 exhibited convulsions during an attempt to sample blood and was euthanized shortly thereafter. No visible lesions were detected at necropsy (Table 5). Control group rat 95A8124 exhibited decreased activity and was later found dead. Necropsy revealed a mass of clotted blood at the surgical incision site, suggesting a leakage of blood at this site. Clotted blood was also found in one other rat in this group, but without any associated clinical signs. Rat 95A8133 from the NaG group exhibited decreased activity (lying on side) and was later euthanized. No gross lesions were found at necropsy. Correct cannula placement within the lumen of the right common carotid artery was confirmed at necropsy in all rats.

Mean body weights decreased between surgery on gestation 8 and the start of dosing on gestation day 10 (Table 6). However, mean body weight loss during this interval was less than 9% in all groups. Control rats then gained an average of 1.3 grams of body weight from gestation day 10 to 11, while the rats in the treated groups exhibited a loss of several grams body weight during this time.

Part I Summary. The following are the salient findings of Part I:

- (1) Doses of 650 mg/kg (8.5 mmol/kg) of GA, 833 mg/kg (8.5 mmol/kg) of NaG or 2500 mg/kg (40.3 mmol/kg) EG resulted in peak blood glycolate concentrations which were within 5% of one another.
- (2) In comparing GA and NaG kinetics, the elimination constants and elimination half lives were essentially identical, and the AUC values were very similar to one another. The major difference between the two curves was the more rapid absorption of NaG, due its SC route of administration. If one accounted for the differences in the initial absorption phase, the remaining portion of the curves would overlap one another almost identically.
- (3) The GA dose caused a mild metabolic acidosis in pregnant rats which was nearly identical to that caused by 2500 mg/kg of EG, while administration of subcutaneous NaG did not induce any detectable acidosis.
- (4) AUC values and serum elimination half-lives were three-fold higher in the EG group compared to those of the GA and NaG groups. These differences were considered potential modifying factors important in the final interpretation of the study data.

Part II

The treatment regimens developed and validated in Part I were then applied in a developmental toxicity study design in which groups of 25 time-mated rats were dosed on gestation days 6-15 with (1) distilled water via gavage, (2) 650 mg/kg/day of GA via gavage, (3) 833 mg/kg/day of NaG via SC injection, or (4) 2500 mg/kg/day of EG via gavage. As mentioned earlier, these dose levels were originally based on a study in male rats indicating that 21% of a 2000 mg/kg IV bolus dose was converted to GA (Marshall, 1982). Parameters of maternal and developmental toxicity were evaluated as detailed in the Materials and Methods section.

Analysis of dosing solutions. All dose solutions assayed from 99-107% of target concentrations and were stable throughout the dosing period (Table 7). As the materials were in solution, homogeneity analyses were not indicated.

In-life observations. Several dams in the 650 mg/kg GA group were noted with mouth breathing, noisy or deep respirations, facial soiling, salivation and/or excessive chromorhinorrhea (Table 8). Three of these dams (96A0857, 96A0874, 96A0878) were sacrificed due to their respiratory difficulties. Another dam (96A0862) was found dead, with no prior clinical symptoms observed. One dam (96A0923) in the EG group exhibited vaginal bleeding on gestation day 19 and 20. The latter dam was found with a completely resorbed litter on gestation day 21, suggesting that the prior vaginal bleeding was related to fetal demise.

Gross pathology - spontaneous deaths/moribund animals. Gross pathological findings among the four GA group dams that either died or were sacrificed in moribund condition included mucoid exudate in the nasal turbinates, soiling of the face/nose region with blood, clear fluid and/or porphyrin, gas in the stomach, congestion of the liver, lungs and kidneys and dilated renal pelvis (Table 9). Based on both the in-life clinical history of respiratory difficulty and the gross pathology findings of the nasal region along with gas in the stomach, the most probable cause of death/moribundity was considered to be obstruction of the nasal turbinates. Considering the acidic nature of the test material and the absence of any findings in the remaining GA rats, these respiratory effects may

have been due to nasal reflux of small amounts of test material as a complication of oral gavage dosing.

Feed consumption. Mean feed consumption values were slightly decreased during the treatment and post-treatment periods (Table 10). Percent decreases from controls ranged from 1-7% for the NaG group, 4-12% for the GA group and 6-13% for the EG group.

Body weights/body weight gains. Maternal body weights were significantly decreased on gestation days 16 and 21 in the GA dams and on gestation days 12, 16 and 21 in the EG group (Table 11). Similarly, body weight gains were significantly decreased for the gestation day 9-12, 12-16, 0-21 and 6-16 intervals in the GA group, and all intervals (except the 0-6 pre-dosing period) in the EG group (Table 12). Body weights and body weight gains in the NaG dams were not significantly different from controls.

Organ weights. Weights of the kidney, a known target organ for EG, were significantly increased in both the EG (absolute and relative weight) and GA (relative weight only) groups (Table 13). Mean absolute and relative kidney weights in the NaG rats were slightly higher than controls, but the differences did not achieve statistical significance. Liver weights were also increased in all three groups, with statistical differences from controls being identified for absolute liver weight in the NaG and EG groups, and for relative liver weights in all three treatment groups.

Gross necropsy - scheduled necropsy. There were no visible lesions observed in any of the dams surviving to the scheduled necropsy (Table 14).

Reproductive/fetal observations made at necropsy. Pregnancy rate across all groups was uniform, with only one of the 100 rats placed on test being found not pregnant (Table 15). There were no effects on the number of corpora lutea or implantations, percent preimplantation loss, or sex ratio. Resorption rate tended to be elevated slightly in all groups relative to controls, but none of the differences reached the predetermined level of statistical significance. While resorption rate in the GA and NaG groups was within the range of historical control, in the EG

group, values for mean resorptions/litter, percent of implantations resorbed and fetuses per litter slightly exceeded the maximum limit of historical control values (Table 16). This suggests that the increased resorption rate in the EG group was due to the test material. Contributing to the elevated resorption rate in the EG group was one totally resorbed litter. Fetal body weights were significantly decreased in all three experimental groups, with this parameter being most severely affected in the EG group, followed by the GA group and then the NaG group. Gravid uterine weights also were significantly decreased in the GA and EG groups.

Fetal morphologic alterations. Treatment with 2500 mg/kg/day of EG resulted in significantly increased incidences of numerous fetal variations and malformations (Table 17), the nature of which was consistent with the previously reported findings of Price et al. (1985) and Neeper-Bradley et al. (1995). The malformations observed in the present study following oral EG exposure consisted primarily of axial skeleton defects (primarily fused, extra, missing or incorrectly paired bones of the vertebral column and ribs), cranial neural tube defects (exencephaly, meningoencephalocele, dilated cerebral ventricles), cranio-facial abnormalities (cleft lip, cleft palate, anophthalmia, microphthalmia) and abdominal wall defects (omphalocele, gastroschisis, umbilical hernia). Limb rotations were also seen, although these appeared in the majority of cases to be related to physical obstructions associated with abdominal wall alterations. A number of isolated visceral malformations occurred in the EG group, but no clear pattern of these alterations was readily identifiable.

In the GA group fetuses, all of the skeletal malformations and 10 of the 11 skeletal variations which were significantly increased following EG treatment were also seen in the GA group, albeit at lesser frequencies (Tables 17, 18). Dilated cerebral ventricles, limb rotations and the abdominal wall defects were additional findings common to both the EG and GA groups. The only malformations found at an increased incidence in the EG group which were not represented in the GA group were several external malformations involving the cranial neural tube and craniofacial region. GA exposure did not result in any unique fetal alterations above and beyond those observed with EG.

NaG treatment caused a statistically significant increase in six skeletal variations, all of which were also seen in the EG group. Also, two axial skeletal malformations consistent with the EG group (hemivertebra and missing ribs) were observed in single NaG group fetuses. Overall, however, the incidence and severity of the fetal alterations in the NaG group was much less than that of either the EG or GA groups. The character and incidence of fetal effects in the NaG group resembled those seen at the LOEL in several other EG developmental toxicity studies (Yin et al., 1986; Neeper-Bradley et al., 1995).

In addressing the primary study objective of determining the relative contributions of the glycolate anion vs. metabolic acidosis in EG developmental toxicity, there are two major conclusions supported by the data. The first conclusion deals with the effects of the glycolate anion *per se*. The data clearly show that some of the developmental effects of EG can be induced by the glycolate anion in the absence of maternal metabolic acidosis. However, significant fetal effects observed under such conditions consisted primarily of decreased fetal body weights and increased incidences of several skeletal variations. This profile of developmental effects is typically seen at the threshold or LOEL of the EG dose response curve as observed in prior developmental toxicity studies (Yin et al., 1986; Neeper-Bradley et al., 1995). This result indicates that metabolic acidosis is not an absolute requirement for these less severe manifestations of developmental toxicity, with glycolate kinetics instead being the major mechanistic determinant for these effects.

The second conclusion regards the role of metabolic acidosis. In contrast to the relatively minor effects caused by NaG, the incidence and severity of developmental effects was much greater in the GA group. Given that serum glycolate levels were very similar following GA and NaG administration, but only GA induced a mild metabolic acidosis, it is concluded that metabolic acidosis is a major exacerbating factor leading to the teratogenic effects of EG at high dose levels. This result was consistent with data obtained by Khera (1991), who greatly ameliorated most of EG's teratogenic effects by co-administering sodium bicarbonate with EG to reduce the degree of acidosis.

A related question for which the current study offers some critical insight is whether or not the evidence is sufficient to conclude that the EG metabolite, GA, is the proximate toxicant for all EG developmental effects *in vivo*. As discussed earlier, exposure of gestation day 10.5 rat embryos in a previous whole rat whole embryo culture study using up to 50 mmol/L EG was essentially without effect, whereas GA at 12.5 mmol/L caused embryonic dysmorphogenesis consistent with EG effects *in vivo* (Carney et al., 1996). Also, numerous studies have shown that the acute toxic effects of EG are due to the GA metabolite and that parent EG has an extremely low intrinsic toxicity (reviewed in Carney, 1994). The present *in vivo* study results indicate that GA is the key metabolite responsible for EG's effects on fetal body weights, axial skeleton variations and malformations, abdominal wall defects and dilated cerebral ventricles, based on the common occurrence of these in both the EG and GA groups. The only effects for which the present study leaves some uncertainty as to the role of parent EG are the external malformations involving the cranial neural tube (meningoencephalocele, exencephaly) and cranio-facial region (cleft lip, cleft palate, anophthalmia, microphthalmia), as these were only seen in the EG group.

There are two possibilities for explaining these results. One, this subset of effects which incidentally, is restricted to the upper extreme of the EG dose response curve (Price et al., 1985; Neeper-Bradley et al., 1995), may be due directly to parent EG. Again, this is highly unlikely given the lack of effects on neural tube and cranio-facial development in whole embryo culture. Furthermore, cranio-facial defects were induced by GA in this system. The alternate hypothesis is that these effects resulted from the three-fold higher AUC_{glycolate} observed after EG dosing vs. dosing with GA. This interpretation is more consistent with the larger body of data on EG and appears to be a much more plausible explanation.

CONCLUSIONS

In summary, the present study incorporated a novel experimental protocol in which treatment with EG, GA or NaG induced very similar peak serum glycolate levels associated either with metabolic acidosis (EG, GA) or without metabolic acidosis (NaG) to determine the relative contributions of these two factors to EG-induced developmental toxicity *in vivo*. Decreased fetal body weights and

increased incidences of six minor skeletal variations were observed with NaG, showing that the glycolate anion is sufficient to cause these most subtle of EG developmental effects in the absence of metabolic acidosis. Treatment with GA resulted in a much greater severity of developmental effects, which also included a variety of malformations characteristic of EG, indicating that metabolic acidosis is a major exacerbating factor leading to *in vivo* teratogenesis following large oral bolus exposures to EG. The high degree of correspondence between the GA and EG fetal alterations supports previous *in vitro* data indicating that EG's developmental effects *in vivo* are due to its metabolite, GA.

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REFERENCES

- Brun-Pascaud, M., Gaudebout, C., Blayo, M. C. and Pocidalò, J. J. (1982). Arterial blood gases and acid-base status in awake rats. Resp. Physiol., 48:45-57.
- Carney, E.W. (1994). An integrated perspective on the developmental toxicity of ethylene glycol. Reproductive Toxicology, 8:99-113.
- Carney, E.W., Liberacki, A., Bartels, M. and Breslin, W. (1996). Identification of proximate toxicant for ethylene glycol developmental toxicity using rat whole embryo culture. Teratology, 53:38-46.
- Dawson, A. B. (1926). A Note on the Staining of the Skeletons of Cleared Specimens with Alizarin Red-S. Stain Tech., 1:123-124.
- Frantz, S. W., Beskitt, J. L., Grosse, C. M., Jensen, C.B., Tallant, M. J., Dietz, F.K. and Ballantyne, B. (1996). Pharmacokinetics of ethylene glycol. 1. Plasma disposition after single intravenous, peroral or percutaneous doses in female Sprague-Dawley rats and CD-1 mice. Drug Metab. Disposition, 24:911-921.
- Gibaldi, M. and Perrier, D. (1982). Pharmacokinetics. Marcel Dekker, Inc., New York, NY.
- Grubbs, F. E. (1969). Procedures for Detecting Outlying Observations in Samples. Technometrics, 11:1-21.
- Haseman, J. K. and Hoel, D. G. (1974). Tables of Gehan's Generalized Wilcoxon Test with Fixed Point Censoring. J. Statist. Comput. Simul., 3:117-135.
- Hewlett, T.P., Jacobsen, D., Collins, T.D. and McMartin, K.E. (1989). Ethylene glycol and glycolate kinetics in rats and dogs. Vet. Human Toxicol., 31:116-120.
- Hollander, M. and Wolfe, D. A. (1973). Nonparametric Statistical Methods. John Wiley and Sons, New York.

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.

Kopf, R., Lorenz, D. and Salewski, E. (1964). [The Effect of Thalidomide on the Fertility of Rats: In an Examination of Two Generations.] - Containing the Procedure for Staining Implantation Sites of Fresh Rat Uteri. Naunyn-Schmiedebergs Arch. Exp. Path. Pharmacol., 247:121-135.

Marshall, T. C. (1982). Dose-dependent disposition of ethylene glycol in the rat after intravenous administration. J. Toxicol. Env. Hlth., 10:397-409.

Miller, R. G. Jr., (1966). Simultaneous Statistical Inference. McGraw-Hill Book Company, Inc., New York.

Munley, S. M. and Hurtt, M. E. (1996). Developmental toxicity study of glycolic acid in rats. Teratology, 53:117 (Abstract).

Neeper-Bradley, T.L., Tyl, R.W., Fisher, L.C., Kubena, M.F., Vrbanic, M.A. and Losco, P.E. (1995). Determination of a no-observed-effect level for developmental toxicity of ethylene glycol administered by gavage to CD rats and CD-1 mice. Toxicol Fundam. Appl., 27:121-130.

Price, C.J., Kimmel, C.A., Tyl, R.W. and Marr, M.C. (1985). The developmental toxicity of ethylene glycol in rats and mice. Toxicol. Appl. Pharmacol., 81:113-127.

Siegel, S. (1956). Non-Parametric Statistics for the Behavioral Sciences. McGraw-Hill Book Company, Inc., New York.

Staples, R. E. (1974). Detection of Visceral Alterations in Mammalian Fetuses. Teratology, 9: 37 (Abstract).

Steel, R. G. D. and Torrie, J. H. (1960). Principles and Procedures of Statistics. McGraw-Hill Book Company, Inc. New York, pp. 101-105 and 111-112.

Wilson, J. G. (1965). Method for Administering Agents and Detecting Malformations in Experimental Animals. In: Teratology: Principles and Techniques (J.G. Wilson and J. Warkany, eds.). University of Chicago Press, Chicago.

Winer, B. J. (1971). Statistical Principles in Experimental Design. 2nd Edition, McGraw-Hill Book Company, Inc., New York.

Yin L, Liu C, Shih L, Po K. (1986). A study of the teratogenic action of ethylene glycol in rats. Chinese J. Prev. Med., 20:289-290.

Figure 1

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

PROPOSED MODE OF ACTION FOR EG INDUCED DEVELOPMENTAL TOXICITY

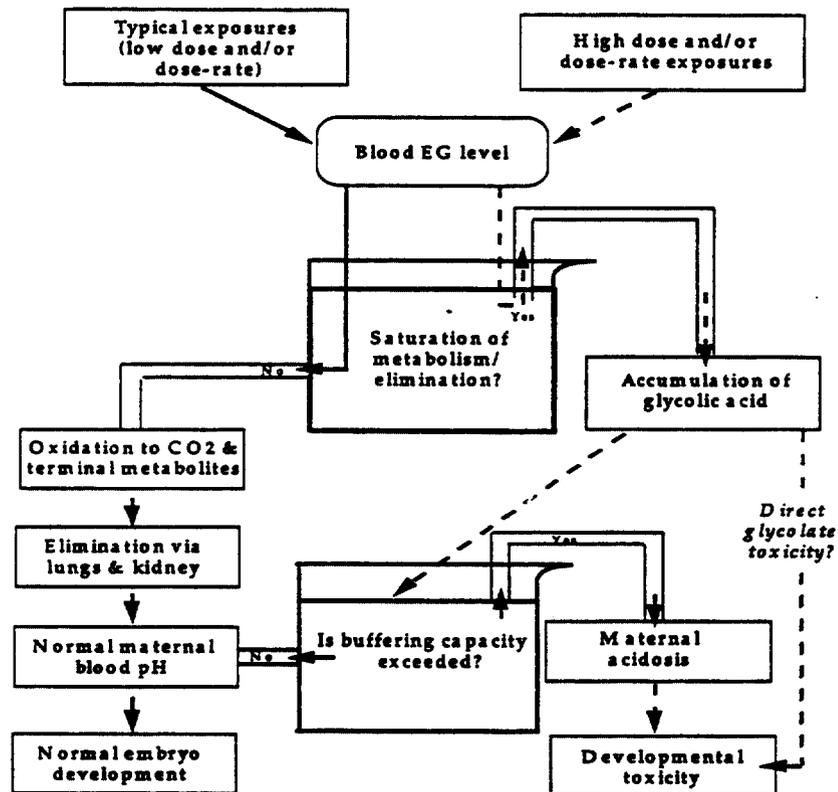


Figure 2

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

MEAN ARTERIAL BLOOD PH CHANGES FOLLOWING SINGLE DOSE OF EG, GA, NaG or Vehicle-Part I

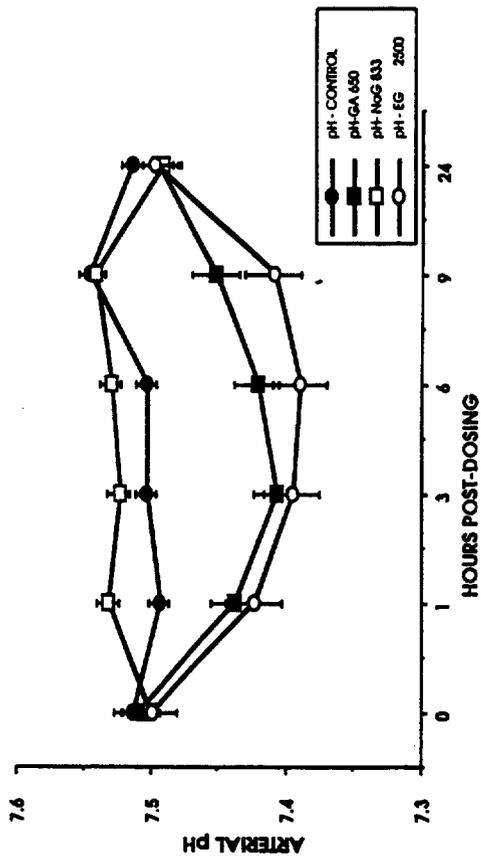
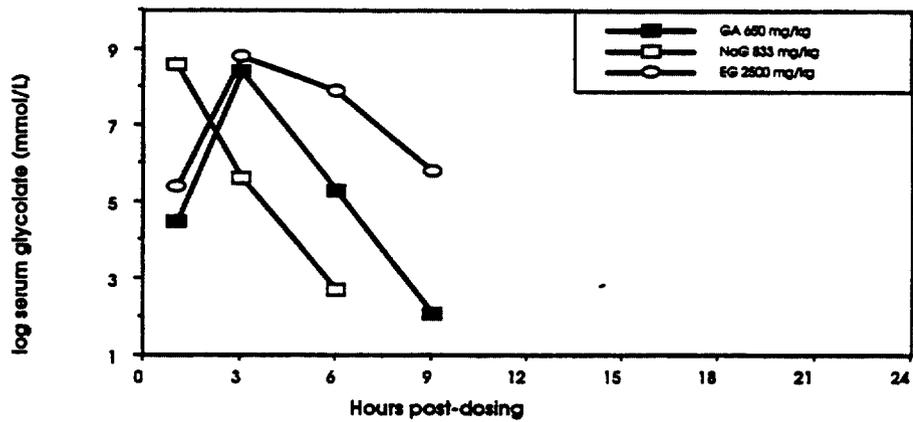


Figure 3

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

SERUM GLYCOLATE IN GD 10 RATS DOSED WITH GA (PO) OR NaG (SC)-PART I



Serum glycolate expressed in mmol/L ($\text{mmol/L} = \frac{\text{ug glycolate}}{\text{ml}} \times \frac{1 \text{ mmol}}{76.05 \text{ mg}} \times \frac{1000 \text{ ml}}{\text{L}} \times \frac{\text{mg}}{1000 \text{ ug}}$)

Where formula wt of glycolic acid=76.05

Table 1

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Acid-Base Values Summary-Part I-^a

GROUP	BLOOD pH						
	0h	1h	3h	6h	9h	24h	
CONTROL	MEAN:	7.514	7.495	7.488	7.503	7.544	7.514
	SD:	0.031	0.044	0.021	0.040	0.029	0.023
	N:	5	5	5	5	3	2
GA 650	MEAN:	7.511	7.437	7.408	7.422	7.451	7.496
	SD:	0.018	0.033	0.049	0.031	0.024	0.049
	N:	5	5	5	5	4	3
NaG 833	MEAN:	7.504	7.533	7.522	7.535	7.546	7.498
	SD:	0.013	0.019	0.017	0.032	0.013	0.050
	N:	4	4	4	4	3	3
EG 2500	MEAN:	7.499	7.424	7.393	7.386	7.413	7.500
	SD:	0.032	0.045	0.046	0.074	0.059	0.032
	N:	5	5	5	5	5	5

CHANGES IN N DUE TO BLOCKED CANNULA OR PREMATURE DEATHS
^a VALUES FROM NON-PREGNANT ANIMALS WERE EXCLUDED

Table 1 (continued)
 ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Acid-Base Values Summary-Part I[ⓐ]

GROUP	ARTERIAL PCO ₂ (mmHg)						
	0h	1h	3h	6h	9h	24h	
CONTROL	MEAN:	33.7	36.5	36.6	34.3	32.1	36.1
	SD:	3.99	4.72	3.99	4.88	3.99	6.79
	N:	5	5	5	5	3	2
GA 650	MEAN:	33.0	32.1	32.2	33.3	33.0	33.9*
	SD:	2.96	2.29	6.11	2.90	2.76	7.85
	N:	5	5	5	5	4	2
NaG 833	MEAN:	34.7	34.2	36.3	35.2	34.0	35.4
	SD:	1.28	2.56	3.48	2.89	0.75	7.66
	N:	4	4	4	4	3	3
EG 2500	MEAN:	36.2	38.3	30.4	30.5	30.0	35.3
	SD:	1.56	9.75	4.20	2.29	3.15	5.38
	N:	5	5	5	5	5	5

CHANGES IN N DUE TO BLOCKED CANNULA OR PREMATURE DEATHS
 * VALUES FROM NON-PREGNANT ANIMALS WERE EXCLUDED
 * ONE SAMPLE ONLY ANALYZED FOR pH DUE TO BLOOD GAS
 PROGRAMMING ERROR

Table 1 (continued)

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Acid-Base Values Summary-Part I⁰

BLOOD BICARBONATE (HCO₃; mmol/L)

GROUP	0h	1h	3h	6h	9h	24h	
CONTROL	MEAN:	27.0	27.9	27.6	26.7	27.6	28.9
	SD:	2.26	1.77	1.88	2.11	2.53	3.96
	N:	5	5	5	5	3	2
GA 650	MEAN:	26.3	21.6	20.1	21.7	22.8	26.6*
	SD:	1.45	0.66	2.14	1.55	1.62	2.47
	N:	5	5	5	5	4	2
NaG 833	MEAN:	27.3	28.7	29.6	29.7	29.4	27.1
	SD:	0.73	1.69	1.89	1.59	1.36	2.96
	N:	4	4	4	4	3	3
EG 2500	MEAN:	28.2	24.6	18.4	18.5	19.4	27.4
	SD:	2.90	3.64	1.50	3.12	3.93	3.25
	N:	5	5	5	5	5	5

CHANGES IN N DUE TO BLOCKED CANNULA OR PREMATURE DEATHS

⁰ VALUES FROM NON-PREGNANT ANIMALS WERE EXCLUDED

* ONE SAMPLE ONLY ANALYZED FOR pH DUE TO BLOOD GAS PROGRAMMING ERROR

Table 2

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Serum Glycolate Values Summary-Part I

SAMPLE I.D.	MEAN CONC. (ug/ml)	STD. DEV.	% RSD	SAMPLE I.D.	MEAN CONC. (ug/ml)	STD. DEV.	% RSD
GA 650 - 0 HR.	N.D.			EG 2500 - 0 HR.	N.D.		
GA 650 - 1 HR.	339	86	25.5%	EG 2500 - 1 HR.	410	109	26.6%
GA 650 - 3 HR.	636	150	23.6%	EG 2500 - 3 HR.	665	36	5.5%
GA 650 - 6 HR.	403	160	39.6%	EG 2500 - 6 HR.	599	67	11.2%
GA 650 - 9 HR.	158	152	96.3%	EG 2500 - 9 HR.	441	274	62.2%
GA 650 - 24 HR.	N.D.			EG 2500 - 24 HR.	N.D.		
NaG 833 - 0 HR.	N.D.						
NaG 833 - 1 HR.	652	70	10.7%				
NaG 833 - 3 HR.	427	90	21.1%				
NaG 833 - 6 HR.	201	117	57.9%				
NaG 833 - 9 HR.	N.D.						

N.D. = NOT DETECTED

Table 3

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS
 Serum Glycolate Pharmacokinetic Values Summary-Part I

Test material	Dose m/kg/day	Route	C _{max}	T _{max}	AUC	K _{elim}	t _{1/2} elim
GA	650	Gavage	8.4	3	55.9	0.231	3
NaG	833	SC	8.6	<1	42.6	0.233	3
EG	2500	Gavage	8.8	3	146.6	0.069	10

C_{max}= Peak concentration (mmol/L)
 T_{max}= Time to Peak Concentration (hours)
 AUC= Area under the curve (mmol L⁻¹ h)
 K_{elim}=Elimination constant
 t_{1/2} elim=Elimination half-life (hours)
 SC=subcutaneous injection

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Table 4

In-Life Observations Summary-Part I-0

SEX:	FEMALES				
MG/KG/DAY:		650 GA	833 NaG	2500 EG	
NUMBER OF ANIMALS OBSERVED:		5	5	5	5
EYES (PHTHISIS BULBI, EYE OPAQUE, ETC.)					
WITHIN NORMAL LIMITS	5	5	5	5	5
FECEES AND URINE (DARK, MUCCOID, REDDISH, ETC.)					
WITHIN NORMAL LIMITS	5	5	5	5	5
GENERAL (COMPLICATIONS OF CANNETER, ETC.)					
WITHIN NORMAL LIMITS	3	5	4	5	5
CONVULSIONS	1	0	0	0	0
DECREASED ACTIVITY	1	0	0	0	0
MORIBUND	0	0	1	0	0
SACRIFICED/FOUND DEAD	2	0	1	0	0
MASS/ NODULE					
WITHIN NORMAL LIMITS	5	5	5	5	5
MOVEMENT/ BEHAVIOR (POSTURE, TREMOR, AGGRESSIVE, ETC.)					
WITHIN NORMAL LIMITS	5	5	5	5	5
RESPIRATION (DEEP, NOISY, SNEEZE, ETC.)					
WITHIN NORMAL LIMITS	5	5	5	5	5
SKIN, FUR, AND MUCOUS MEMBRANES					
WITHIN NORMAL LIMITS	5	5	5	5	5

0 DATA ARE THE NUMBER OF ANIMALS WITH THE SPECIFIED OBSERVATION

Table 5

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Necropsy Observations Summary-Part I

TEST MATERIAL:	FEMALES			
	CONTROL	GA	NaG	EG
<u>MG/KG:</u>	<u>0</u>	<u>650</u>	<u>833</u>	<u>2500</u>
NUMBER OF ANIMALS OBSERVED	5	5	5	5
GENERAL				
NO VISIBLE LESIONS	4	5	4	5
SURGERY INCISION SITE: CLOTTED BLOOD	1	0	1	0

Table 6

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Gestation Body Weights-Part I

<u>GROUP</u>	<u>PRE-SURGERY</u>			<u>TERMINAL</u>	<u>BODY WT GAIN</u>	
	<u>GD 8 BODY WT (G)</u>	<u>GD 10 BODY WT (G)</u>	<u>BODY WT (G)</u>	<u>GD 8 - 10 (G)</u>	<u>GD 10-TERM (G)</u>	
CONTROL	MEAN:	279.8	255.4	260.7	-24.4	1.3
	SD:	26.2	34.9	48.7	11.1	1.2
	N:	5	5	3	5	3
GA 650	MEAN:	273.0	254.6	245.0	-18.4	-9.6
	SD:	35.9	31.5	27.1	15.9	8.9
	N:	5	5	5	5	5
NaG 833	MEAN:	283.3	264.5	257.3	-18.8	-4.7
	SD:	39.7	28.3	34.1	12.0	1.2
	N:	4	4	3	4	3
EG 2500	MEAN:	247.6	226.6	220.6	-21.0	-6.0
	STDEV:	35.3	38.3	40.5	14.7	9.7
	N:	5	5	5	5	5

NON-PREGNANT ANIMALS EXCLUDED FROM CALCULATION OF MEANS AND STANDARD DEVIATIONS
 BLOCKED CANNULA ANIMALS INCLUDED IN CALCULATION OF MEANS AND STANDARD DEVIATIONS

Table 7

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS
 Concentrations of Test Material In Dosing Solutions-Part II

TEST MATERIAL: MG/KG/DAY:	CONTROL	GA	NaG	EG
	0	650	833	2500
Target concentration (mg/ml)	0	163	212.5	625
<u>Initial analysis (2/12/96):</u> Concentration (mg/ml)	NA	175	211	625
% of target	-	107	99	100
<u>Final analysis (3/8/96):</u> Concentration (mg/ml)	ND	161	219	627
% of target	-	99	103	100

NA = CONTROL SOLUTION NOT ANALYZED AT INITIAL ANALYSIS
 ND = NOT DETECTED (ESTIMATED LIMITS OF DETECTION WERE 0.002 MG/ML,
 GA, 0.003 MG/ML, AND 0.01 MG/ML FOR GA, NaG, AND EG, RESPECTIVELY.

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Table 8

In-Life Observations Summary-Part II-θ

SEX MG/KG/DAY NUMBER OF ANIMALS OBSERVED	FEMALES			
	0 25	650GA 25	833NAG 25	2500EG 25
EYES (PHTHISIS BULBI, EYE OPAQUE, ETC.)	25	24	25	25
WITHIN NORMAL LIMITS	0	1	0	0
EXCESSIVE CHROMODACRYORRHEA	25	25	25	25
FECES AND URINE (DARK, MUCCOID, REDDISH, ETC.)	25	25	25	25
WITHIN NORMAL LIMITS	25	25	25	25
GENERAL (SWELLING, OBSE, NASAL DISCHARGE, ETC.)	25	22	25	24
WITHIN NORMAL LIMITS	0	2	0	0
SALIVATION	0	2	0	0
EXCESSIVE CHROMORRHINORRHEA	0	0	0	1
VAGINAL BLEEDING	0	0	0	0
ALL SWELLINGS	23	25	25	25
MASS/NODULE	1	0	0	0
WITHIN NORMAL LIMITS	1	0	0	0
MASS/NODULE, TAIL (WART-LIKE)	2	0	0	0
MASS/NODULE, HIP (ABSCESSES)	0	0	0	0
ALL MASS/MODULES	25	25	25	25
MOVEMENT/BEHAVIOR (POSTURE, TREMOR, AGGRESSIVE, ETC.)	25	22	25	25
WITHIN NORMAL LIMITS	0	2	0	0
RESPIRATION (DEEP, NOISY, SNEEZE, ETC.)	25	22	25	25
WITHIN NORMAL LIMITS	0	0	0	0
MOUTH BREATHING	0	3	0	0
RESPIRATION NOISY	0	1	0	0
RESPIRATION DEEP	25	24	24	25
SKIN, FUR AND MUCCOUS MEMBRANES	0	1	0	0
WITHIN NORMAL LIMITS	0	0	0	0
FACIAL SOILING	0	0	1	0
ABRASION	0	4	0	0
SACRIFICED/FOUND DEAD	0	0	0	0

θ DATA ARE THE NUMBER OF ANIMALS WITH THE SPECIFIED OBSERVATION.

Table 9

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Gross Necropsy--Spontaneous/Moribund Deaths-Part II-θ

SEX	FEMALES		
	DOSE IN MG/KG/DAY	NUMBER OF RATS EXAMINED	WITHIN NORMAL LIMITS.
DIGESTIVE TRACT GAS:	0	650	833
	4	4	0
EXTERNAL AND SKIN WITHIN NORMAL LIMITS.	0	1	1
	4	1	1
BLOOD, MUZZLE: FACIAL SOILING - CLEAR: FACIAL SOILING - PORPHYRIN:	0	1	1
	4	1	1
GENERAL WITHIN NORMAL LIMITS. CONGESTION OF LIVER, LUNGS AND KIDNEYS:	0	3	3
	4	1	1
KIDNEYS DILATED, PELVIS, UNILATERAL:	0	3	3
	4	1	1
NASAL TISSUES WITHIN NORMAL LIMITS. EXUDATE - MUCCOID: EXUDATE - MUCCOID, LUMEN:- MODERATE	0	2	2
	4	1	1
STOMACH WITHIN NORMAL LIMITS. GAS:	0	3	3
	4	1	1

θ DATA ARE THE NUMBER OF ANIMALS WITH THE SPECIFIED OBSERVATION.
 - INDICATES NOT APPLICABLE.

Table 9 (continued)

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Gross Necropsy-Spontaneous/Moribund Deaths-Part II-θ

SEX	FEMALES			
	0	650	833	2500
<u>DOSE IN MG/KG/DAY</u>	0	650	833	2500
<u>NUMBER OF RATS EXAMINED</u>	0	4	0	0
<u>UTERUS</u>				
WITHIN NORMAL LIMITS	-	0	-	-
PREGNANT-ALL FETUS(ES) APPEAR NORMAL:	-	3	-	-
PREGNANT-NORMAL APPEARING FETUS(ES) WITH RESORPTIONS AND/OR	-			
DEAD FETUS(ES) :	-	1	-	-

θ DATA ARE THE NUMBER OF ANIMALS WITH THE SPECIFIED OBSERVATION.
 - INDICATES NOT APPLICABLE.

Table 10

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYOCLATE ANION AND METABOLIC ACIDOSIS

Feed Consumption Summary-Part II-θ
 DAYS OF GESTATION

DOSE MG/KG/DAY	3-6	6-9	9-12	12-16	16-19	19-21
0	MEAN 21.2 S.D. 2.7 N= 24	MEAN 22.6 S.D. 2.1 N= 24	MEAN 24.3 S.D. 2.4 N= 24	MEAN 25.5 S.D. 2.2 N= 24	MEAN 27.7 S.D. 2.1 N= 24	MEAN 25.9 S.D. 1.5 N= 24
GA 650	MEAN 21.2 S.D. 1.6 N= 25	MEAN 21.6 S.D. 2.7 N= 23	MEAN 21.4 S.D. 4.9 N= 23	MEAN 23.4 S.D. 2.8 N= 21	MEAN 25.5 S.D. 2.5 N= 21	MEAN 24.8 S.D. 2.4 N= 20
NaG 833	MEAN 21.3 S.D. 1.5 N= 25	MEAN 21.1 S.D. 1.3 N= 25	MEAN 22.7 S.D. 1.9 N= 25	MEAN 24.2 S.D. 2.1 N= 25	MEAN 27.4 S.D. 2.3 N= 25	MEAN 25.3 S.D. 2.0 N= 25
EG 2500	MEAN 21.4 S.D. 1.8 N= 24	MEAN 19.7 S.D. 2.2 N= 24	MEAN 20.9 S.D. 2.1 N= 24	MEAN 22.7 S.D. 1.8 N= 24	MEAN 25.8 S.D. 2.3 N= 24	MEAN 24.4 S.D. 2.3 N= 24

θ ANIMALS WITHOUT VIABLE FETUSES ON DAY 21 WERE EXCLUDED FROM CALCULATION OF MEANS AND STANDARD DEVIATIONS

Table 11

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Gestation Body Weights Summary-Part II-e
 DAY OF GESTATION

DOSE MG/KG/DAY	DAY OF GESTATION									
	0	6	9	12	16	21	21	21	21	21(C)
0	MEAN	219.6	251.1	269.6	288.9	320.0	394.0	394.0	294.1	294.1
	S.D.	7.5	13.2	14.6	16.9	18.9	23.2	23.2	17.8	17.8
	N=	24	24	24	24	24	24	24	24	24
GA 650	MEAN	220.8	251.4	265.9	280.1	302.1*	371.3*	371.3*	287.9	287.9
	S.D.	7.2	9.3	15.3	16.6	17.9	23.1	23.1	15.6	15.6
	N=	25	25	23	23	21	21	21	21	21
NaG 833	MEAN	221.8	252.9	268.9	287.5	317.5	387.9	387.9	294.6	294.6
	S.D.	8.1	8.9	9.1	11.7	15.4	22.1	22.1	14.4	14.4
	N=	25	25	25	25	25	25	25	25	25
EG 2500	MEAN	220.7	251.9	264.3	277.3*	301.8*	361.5*	361.5*	291.7	291.7
	S.D.	7.8	9.8	11.3	14.0	16.2	25.5	25.5	13.0	13.0
	N=	24	24	24	24	24	24	24	24	24

* STATISTICALLY DIFFERENT FROM CONTROL MEAN BY DUNNETT'S TEST, ALPHA = 0.05.
 † ANIMALS WITHOUT VIABLE FETUSES ON DAY 21 WERE EXCLUDED FROM ANALYSIS.
 21(C) = DAY 21 BODY WEIGHT - GRAVID UTERUS WEIGHT

Table 12

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Gestation Body Weight Gains Summary-Part II-@

DOSE MG/KG/DAY	DAY OF GESTATION											
	0-6	6-9	9-12	12-16	16-21	16-21	6-16	6-16	0-21	0-21	0-21	0-21
0	MEAN	31.5	18.5	19.4	31.0	74.1	68.9	174.4				
	S.D.	9.8	5.7	6.1	5.4	8.0	11.3	19.9				
	N=	24	24	24	24	24	24	24	24	24	24	24
GA 650	MEAN	30.6	14.3	14.2*	22.1\$	69.2	49.9*	150.3*				
	S.D.	5.8	9.2	9.4	14.6	12.6	13.1	21.4				
	N=	25	23	23	21	21	21	21	21	21	21	21
NaG 833	MEAN	31.2	16.0	18.6	30.0	70.4	64.6	166.2				
	S.D.	8.5	6.5	5.9	7.9	8.7	12.1	19.9				
	N=	25	25	25	25	25	25	25	25	25	25	25
EG 2500	MEAN	31.2	12.4*	13.1*	24.5\$	59.7*	49.9*	140.8*				
	S.D.	6.6	4.9	4.9	7.0	10.8	10.6	22.0				
	N=	24	24	24	24	24	24	24	24	24	24	24

* STATISTICALLY DIFFERENT FROM CONTROL MEAN BY DUNNETT'S TEST, ALPHA = 0.05.
 \$ STATISTICALLY DIFFERENT FROM CONTROL MEAN BY WILCOXON'S TEST, ALPHA = 0.05.
 @ ANIMALS WITHOUT VIABLE FETUSES ON DAY 21 WERE EXCLUDED FROM ANALYSIS

Table 13

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Organ And Organ/Body Weight Ratios Summary-Part II-θ

DOSE MG/KG/DAY		BODY			KIDNEYS			LIVER		
		WT. (G)	(G)	(G/100)	(G)	(G/100)	(G)	(G/100)		
0	MEAN	394.0	1.918	0.487	13.739	3.486				
	S.D.	23.2	0.215	0.048	1.260	0.239				
	N=	24	24	24	24	24				
GA 650	MEAN	371.3*	1.958	0.528*	14.675	3.952*				
	S.D.	23.1	0.157	0.044	1.567	0.343				
	N=	21	21	21	21	21				
NAG 833	MEAN	387.9	1.970	0.509	15.042*	3.877*				
	S.D.	22.1	0.139	0.034	1.555	0.334				
	N=	25	25	25	25	25				
EG 2500	MEAN	361.5*	2.080*	0.577*	14.934*	4.140*				
	S.D.	25.5	0.213	0.067	1.360	0.375				
	N=	24	24	24	24	24				

* STATISTICALLY DIFFERENT FROM CONTROL MEAN BY DUNNETT'S TEST, ALPHA = 0.05.
θ ANIMALS WITHOUT VIABLE FETUSES ON DAY 21 WERE EXCLUDED FROM ANALYSIS

Table 14

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
ON THE ROLE OF GLYOXALATE ANION AND METABOLIC ACIDOSIS

Gross Necropsy-Scheduled Sacrifice-Part II- θ

SEX	FEMALES			
	GA	NaG	EG	
TEST MATERIAL DOSE IN MG/KG/DAY	0	650	833	2500
NUMBER OF RATS EXAMINED	24	21	25	25

ALL OTHER TISSUES (COMPLETE NECROPSY PERFORMED)
WITHIN NORMAL LIMITS.

θ DATA ARE THE NUMBER OF ANIMALS WITH THE SPECIFIED OBSERVATION.

Table 15

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Observations Made At The Time Of Necropsy Summary-Part II

MG/KG/DAY:	0	GA 650	NaG 833	EG 2500
NUMBER BRED	25	25	25	25
NO. PREGNANT (%) ^a	24 (96)	25 (100)	25 (100)	25 (100)
NUMBER OF DEATHS/MORTIBUND	0	4	0	0
NUMBER OF ABORTIONS	0	0	0	0
DELIVERED EARLY	0	0	0	0
TOTALLY RESORBED LITTERS	0	0	0	1
PREGNANCIES DETECTED BY STAINING ^b	0/1	0/0	0/0	0/0
LITTERS WITH ≥ 1 VIABLE FETUSES	24	21	25	24
MEAN CORPORA LUTEA/DAM ± S.D.	15.4 ± 2.9	15.0 ± 3.9	15.3 ± 2.1	15.9 ± 2.8
MEAN IMPLANTATIONS/DAM ± S.D.	13.4 ± 1.9	13.4 ± 3.2	13.1 ± 1.5	13.6 ± 2.0
MEAN & PREIMPLANT. LOSS/LITTER ± S.D.	11.6 ± 1.9	11.5 ± 17.5	13.3 ± 11.7	13.5 ± 12.0
MEAN FETUSES/LITTER ± S.D.	13.0 ± 1.9	12.6 ± 3.2	12.6 ± 1.5	11.4 ± 3.5
MEAN RESORPTIONS/LITTER ± S.D. ^c	0.3 ± 0.6	0.8 ± 1.1	0.6 ± 0.9	2.2 ± 3.4
& IMPLANTATIONS RESORBED	2.5 (8/321)	5.7 (16/281)	4.3 (14/328)	15.9 (54/340)
& LITTERS WITH RESORPTIONS	29.2 (7/24)	47.6 (10/21)	36.0 (9/25)	64.0 (16/25)
RESORPTIONS/LITTER WITH RESORPTIONS	1.2 (6/5)	2.0 (8/4)	1.5 (3/2)	1.8 (24/13)
SEX RATIO (M:F)	49.6:50.4	50.0:50.0	45.2:54.8	54.7:45.3
FETAL BODY WEIGHT (G)	5.53 ± 0.30	4.57 ± 0.33**	5.07 ± 0.20**	3.75 ± 0.58**
GRAVID UTERINE WEIGHT (G)	99.90 ± 10.36	83.43 ± 20.18**	93.33 ± 10.90	69.80 ± 17.72**

** STATISTICALLY DIFFERENT FROM CONTROL BY CENSORED WILCOXON TEST (ALPHA = 0.01)
 *** STATISTICALLY DIFFERENT FROM CONTROL BY DUNNETT'S TEST (ALPHA = 0.05)
 a DEFINED AS ANY ANIMAL WITH IMPLANTATIONS, INCLUDING THOSE DETECTED BY SODIUM SULFIDE STAINING
 b NO. WITH IMPLANTATIONS DETECTED BY SODIUM SULFIDE STAINING/TOTAL NUMBER STAINED
 c NO STATISTICAL ANALYSIS

Table 16

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Historical Control-Observations Made At Time Of Necropsy

STUDY #	1	2	3	4	5
DATE	Sep-88	May-89	Aug-90	Aug-91	Nov-91
ROUTE	DIET	GAVAGE	DIET	DERMAL	GAVAGE
# BREED	29	30	30	45	30
# PREGNANT	23	25	29	43	28
% PREGNANT	79.3	83.3	96.7	95.6	93.3
# FEMALES DEAD	0	0	0	0	0
% FEMALES DEAD (#DEAD/#BREED)	0	0	0	0	0
# PREGNANT W/STAIN	0	0	1	0	1
# ABORTED	0	0	0	0	0
% ABORTED	0	0	0	0	0
(#ABORTED/#PREGNANT)	0	0	0	0	0
# DELIVERED EARLY	0	0	0	0	0
% DELIVERED EARLY	0	0	0	0	0
(#DEL.EARLY/#PREG)	0	0	0	0	0
TOTALLY RESORBED LITTERS	0	0	1	0	0
# LITTERS	23	25	28	43	27
# FETUSES	269	378	364	636	438
CORPORA LUTEA/DAM	16	18.3	17	18.7	19.7
IMPLANTATION SITES/DAM	13.6	16.1	14.5	15.5	16.8
% PRE-IMPLANTATION LOSS	13.1	12	14.6	15.6	16.3
FETUSES/LITTER	11.7	15.1	13	14.8	16.2
RESORPTIONS/LITTER	1.9	1	1.5	0.7	0.6
# IMPLANTATIONS RESORBED	44	25	41	29	16
% IMPLANTATIONS RESORBED	14.1	6.2	10.1	4.4	3.5
# LITTERS WITH RESORPTIONS	15	14	21	22	10
% LITTERS WITH RESORPTIONS	65.2	56	75	51.2	37
RESORPTIONS/LITTERS W/	2.9	1.8	2	1.3	1.6
RESORPTIONS					
# DEAD FETUSES	0	0	0	0	0
% DEAD FETUSES	0	0	0	0	0
SEX RATIO (%M:%F)	50:50	45:55	50:50	50:50	47:53
AVG FETAL BODY WEIGHT (G)	5.13	5.28	5.03	5.25	5.6
GRAVID UTERINE WEIGHT (G)	85.39	109.49	92.9	107.1	121.4

Table 16 (continued)

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

STUDY #	Historical Control-Observations Made At Time Of Necropsy				MINIMUM	MAXIMUM	AVERAGE
	6	7	8				
DATE	Oct-92	Dec-92	Feb-93				
ROUTE	INHALATION	GAVAGE	GAVAGE				
# BRED	30	30	30	29	45	31.75	
# PREGNANT	26	28	27	23	43	28.63	
% FEMALES DEAD	86.7	93.3	90	79.3	96.7	89.78	
# FEMALES DEAD (#DEAD/#BRED)	0	0	0	0	0	0.00	
# PREGNANT W/ STAIN	0	0	0	0	1	0.00	
# ABORTED	0	0	0	0	0	0.25	
# ABORTED (#ABORTED/#PREGNANT)	0	0	0	0	0	0.00	
# DELIVERED EARLY	1	0	0	0	1	0.13	
# DELIVERED EARLY (#DEL.EARLY/#PREG)	4	0	0	0	4	0.50	
TALLY RESORBED LITTERS	0	0	0	0	1	0.13	
# LITTERS	26	28	27	23	43	28.38	
# FETUSES	394	445	437	269	636	420.13	
CORPORA LUTEA/DAM	20.4	19.2	18.3	16	20.4	18.45	
IMPLANTATION SITES/DAM	16.5	16.7	17.5	13.6	17.5	15.90	
% PRE-IMPLANTATION LOSS	17.7	13	5.9	5.9	17.7	13.53	
FETUSES/LITTER	15.2	15.9	16.2	11.7	16.2	14.76	
RESORPTIONS/LITTER	1.4	0.8	1.3	0.6	1.9	1.15	
# IMPLANTATIONS RESORBED	36	22	36	16	44	31.13	
% IMPLANTATIONS RESORBED	8.4	4.7	7.6	3.5	14.1	7.38	
# LITTERS WITH RESORPTIONS	15	14	22	10	22	16.63	
% LITTERS WITH RESORPTIONS	57.7	50	81.5	37	81.5	59.20	
RESORPTIONS/LITTERS W/ RESORPTIONS	2.4	1.6	1.6	1.3	2.9	1.90	
# DEAD FETUSES	0	0	0	0	0	0.00	
# DEAD FETUSES	0	0	0	0	0	0.00	
SEX RATIO (%M:%F)	48:52	51:49	50:50	-	-	-	
AVG FETAL BODY WEIGHT (G)	5.48	5.39	5.3	5.03	5.6	5.31	
GRAVID UTERINE WEIGHT (G)	112.51	119.23	119.2	85.39	121.4	108.40	

Table 17

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Incidence of Fetal Alterations Summary-Part II

MG/KG/DAY	0		GA 650		NaG 833		EG 2500	
	NUMBER OF FETUSES (NUMBER OF LITTERS)	EXAMINED	NUMBER OF FETUSES (NUMBER OF LITTERS)	EXAMINED	NUMBER OF FETUSES (NUMBER OF LITTERS)	EXAMINED	NUMBER OF FETUSES (NUMBER OF LITTERS)	EXAMINED
EXTERNAL EXAMINATION	313 (24)	286 (24)	264 (21)	314 (25)	151 (24)	151 (24)	135 (24)	135 (24)
VISCERAL EXAMINATION	161 (24)	137 (21)	137 (21)	163 (25)	151 (24)	151 (24)	135 (24)	135 (24)
SKELETAL EXAMINATION	152 (24)	128 (21)	128 (21)	151 (25)	151 (24)	151 (24)	135 (24)	135 (24)
PERCENT AFFECTED (NUMBER AFFECTED)								
EXTERNAL OBSERVATIONS								
HEAD & NEURAL TUBE								
- MENINGOENCEPHALOCELE ⁺	F ⁺ L ⁺	0.3 (1) 4.2 (1)	0 0	0 0	0 0	0 0	0 0	3.5 (10) 25.0 (6)*
- EXENCEPHALY ⁺	F L	0 0	0 0	0 0	0 0	0 0	0 0	5.2 (15) 25.0 (6)*
- CLEFT LIP ⁺	F L	0 0	0 0	0 0	0 0	0 0	0 0	3.5 (10) 29.2 (7)*
- CLEFT PALATE ⁺	F L	0 0	0 0	0 0	0 0	0 0	0 0	2.8 (8) 29.2 (7)*
- HYPOPLASTIC UPPER JAW ⁺ (HYPOGNATHIA)	F L	0 0	0 0	0 0	0 0	0 0	0 0	0.7 (2) 8.3 (2)
ABDOMINAL WALL								
- OMPHALOCELE	F L	0 0	0 0	0 0	0 0	0 0	0 0	9.8 (28) 54.2 (13)*
- GASTROSCHISIS ⁺	F L	0 0	0 0	0 0	0 0	0 0	0 0	0.3 (1) 4.2 (1)
- UMBILICAL HERNIA ⁺	F L	0 0	0.4 (1) 4.8 (1)	0 0	0 0	0 0	0 0	1.7 (5) 16.7 (4)

Table 17 (continued)

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Incidence Of Fetal Alterations Summary-Part II

MG/KG/DAY	0		GA 650		NaG 833		EG 2500	
	NUMBER OF FETUSES (NUMBER OF LITTERS) EXAMINED	PERCENT AFFECTED (NUMBER AFFECTED)	NUMBER OF FETUSES (NUMBER OF LITTERS) EXAMINED	PERCENT AFFECTED (NUMBER AFFECTED)	NUMBER OF FETUSES (NUMBER OF LITTERS) EXAMINED	PERCENT AFFECTED (NUMBER AFFECTED)	NUMBER OF FETUSES (NUMBER OF LITTERS) EXAMINED	PERCENT AFFECTED (NUMBER AFFECTED)
EXTERNAL EXAMINATION	313 (24)		264 (21)		314 (25)		286 (24)	
VISCERAL EXAMINATION	161 (24)		137 (21)		163 (25)		151 (24)	
SKELETAL EXAMINATION	152 (24)		128 (21)		151 (25)		135 (24)	
LIMBS & DIGITS								
- LIMB ROTATION ⁺	F 0	0.4 (1)	F 0	0	F 0	0	F 0	5.2 (15)
	L 0	4.8 (1)	L 0	0	L 0	0	L 0	16.7 (4)
- CLUB FOOT ⁺	F 0	0	F 0	0	F 0	0	F 0	0.7 (2)
	L 0	0	L 0	0	L 0	0	L 0	8.3 (2)
- OLIGODACTYLY ⁺	F 0	0	F 0	0	F 0	0	F 0	0.3 (1)
	L 0	0	L 0	0	L 0	0	L 0	4.2 (1)
TAIL								
- ACAUDIA ⁺	F 0	0.4 (1)	F 0	0.4 (1)	F 0	0.3 (1)	F 0	0
	L 0	4.8 (1)	L 0	4.8 (1)	L 0	4.0 (1)	L 0	0
- RUDIMENTARY TAIL ⁺	F 0	0	F 0	0	F 0	1.0 (3)	F 0	0.3 (1)
	L 0	0	L 0	0	L 0	12.0 (3)	L 0	4.2 (1)
VISCERAL OBSERVATIONS								
DIAPHRAGM								
- HERNIA ⁺	F 0	0.7 (1)	F 0	0.7 (1)	F 0	0	F 0	0.7 (1)
	L 0	4.8 (1)	L 0	4.8 (1)	L 0	0	L 0	4.2 (1)
LUNGS								
- HYPOPLASTIC LUNG LOBE ⁺	F 0	0	F 0	0	F 0	0	F 0	0.7 (1)
	L 0	0	L 0	0	L 0	0	L 0	4.2 (1)

Table 17 (continued)

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Incidence Of Fetal Alterations Summary-Part II

MG/KG/DAY	0		GA 650		NaG 833		EG 2500	
	NUMBER OF FETUSES (NUMBER OF LITTERS)	EXAMINED	NUMBER OF FETUSES (NUMBER OF LITTERS)	EXAMINED	NUMBER OF FETUSES (NUMBER OF LITTERS)	EXAMINED	NUMBER OF FETUSES (NUMBER OF LITTERS)	EXAMINED
EXTERNAL EXAMINATION	313 (24)		264 (21)		314 (25)		286 (24)	
VISCERAL EXAMINATION	161 (24)		137 (21)		163 (25)		151 (24)	
SKELETAL EXAMINATION	152 (24)		128 (21)		151 (25)		135 (24)	
PERCENT AFFECTED (NUMBER AFFECTED)								
GREAT VESSELS								
- INTERRUPTED AORTIC ARCH	F	0	0	0	0	0	0.7 (1)	
	F	0	0	0	0	0	4.2 (1)	
- RIGHT SIDED AORTIC ARCH	F	0	0	0	0	0	0.7 (1)	
	L	0	0	0	0	0	4.2 (1)	
- TRANSPOSITION	F	0	0	0	0	0	0.7 (1)	
	L	0	0	0	0	0	4.2 (1)	
ADRENAL	F	0	0	0	0	0	2.0 (3)	
- ECTOPIC	L	0	0	0	0	0	8.3 (2)	
- REDDENED	F	0	0	0	0	0	0.7 (1)	
	L	0	0	0	0	0	4.2 (1)	
KIDNEY	F	0	0	0	0	0	2.0 (3)	
- ECTOPIC	L	0	0	0	0	0	8.3 (2)	
- MISSING	F	0	0	0	0	0	0.7 (1)	
	L	0	0	0	0	0	4.2 (1)	
- HYDRONEPHROSIS	F	0	0	0	0	0	1.3 (2)	
	L	0	0	0	0	0	8.3 (2)	
URETER	F	0	0.7 (1)	0	0	0	0	0
- HYDROURETER	L	0	4.8 (1)	0	0	0	0	0
- RETROCAVAL URETER	F	0	0	0	0	0	0.7 (1)	
	L	0	0	0	0	0	4.2 (1)	

Table 17 (continued)

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Incidence Of Fetal Alterations Summary-Part II

MG/KG/DAY	0	GA 650	NaG 833	EG 2500
	NUMBER OF FETUSES (NUMBER OF LITTERS) EXAMINED			
EXTERNAL EXAMINATION	313 (24)	264 (21)	314 (25)	286 (24)
VISCERAL EXAMINATION	161 (24)	137 (21)	163 (25)	151 (24)
SKELETAL EXAMINATION	152 (24)	128 (21)	151 (25)	135 (24)
		PERCENT AFFECTED (NUMBER AFFECTED)		
SPLEEN				
- AGENESIS ⁺	F 0	0	0	1.3 (2)
	L 0	0	0	8.3 (2)
BOVIN'S HEAD EXAM				
-DILATED CEREBRAL VENTRICLES	F 0	5.1 (7)	0	15.9 (24)
	L 0	19.0 (4)	0	33.3 (8) *
-ANOPHTHALMIA ⁺	F 0	0	0	3.3 (5)
	L 0	0	0	16.7 (4)
-MICROPHTHALMIA ⁺	F 0	0	0	2.0 (3)
	L 0	0	0	12.5 (3)
SKELETAL OBSERVATIONS				
SKULL				
- D.O.	F 8.6 (13)	11.7 (15)	23.8 (36)	23.7 (32)
	L 29.2 (7)	52.4 (11)	60.0 (15) *	62.5 (15) *
- EXTRA SITE OF OSSIFICATION	F 0	0.8 (1)	0	17.0 (23)
	L 0	4.8 (1)	0	45.8 (11) *

Table 17 (continued)

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Incidence of Fetal Alterations Summary-Part II

MG/KG/DAY	0		GA 650		NaG 833		EG 2500	
	NUMBER OF FETUSES (NUMBER OF LITERS) EXAMINED							
EXTERNAL EXAMINATION	313 (24)	264 (21)	314 (25)	286 (24)				
VISCERAL EXAMINATION	161 (24)	137 (21)	163 (25)	151 (24)				
SKELETAL EXAMINATION	152 (24)	128 (21)	151 (25)	135 (24)				
	PERCENT AFFECTED (NUMBER AFFECTED)							
VERTEBRAE								
- HEMIVERTEBRA ^{+b}	F 0	23.8 (30)	0.7 (1)	60.0 (81)				
	L 0	71.4 (15)*	4.0 (1)	95.8 (23)*				
- EXTRA ^{+b}	F 0	0.8 (1)	0	5.9 (8)				
	L 0	4.8 (1)	0	29.2 (7)*				
- MISSING ^{+b}	F 0	5.6 (7)	0	24.4 (33)				
	L 0	28.6 (6)*	0	62.5 (15)*				
- FUSED ^{ab}	F 0	3.2 (4)	0	36.3 (49)				
	L 0	19.0 (4)	0	75.0 (18)*				
- CERVICAL- D.O.	F 0	0	0	0.7 (1)				
	L 0	0	0	4.2 (1)				
- THORACIC- D.O.	F 0	0	0	1.5 (2)				
	L 0	0	0	8.3 (2)				
- LUMBAR- D.O. ^b	F 0	0	0	8.1 (11)				
	L 0	0	0	33.3 (8)*				
- IRREG PATTERN OSSIFICATION	F 0	0.8 (1)	0	0.7 (1)				
	L 0	4.8 (1)	0	4.2 (1)				
- MISALIGNED	F 0	0	0	1.5 (2)				
	L 0	0	0	8.3 (2)				

Table 17 (continued)

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Incidence Of Fetal Alterations Summary-Part II

MG/KG/DAY	0		GA 650		NaG 833		EG 2500	
	NUMBER OF FETUSES (NUMBER OF LITTERS)	EXAMINED	NUMBER OF FETUSES (NUMBER OF LITTERS)	EXAMINED	NUMBER OF FETUSES (NUMBER OF LITTERS)	EXAMINED	NUMBER OF FETUSES (NUMBER OF LITTERS)	EXAMINED
EXTERNAL EXAMINATION	313 (24)		264 (21)		314 (25)		286 (24)	
VISCERAL EXAMINATION	161 (24)		137 (21)		163 (25)		151 (24)	
SKELETAL EXAMINATION	152 (24)		128 (21)		151 (25)		135 (24)	
PERCENT AFFECTED (NUMBER AFFECTED)								
CENTRA								
- CERVICAL-D.O.	F	32.2 (49)	89.8 (115)		92.7 (140)		97.0 (131)	
	L	75.0 (18)	95.2 (20)*		100.0 (25)*		100.0 (24)*	
- THORACIC-D.O.	F	2.0 (3)	71.9 (92)		39.7 (60)		97.8 (132)	
	L	12.5 (3)	90.5 (19)*		8.0 (22)*		100.0 (24)*	
- LUMBAR-D.O. ^b	F	0	38.9 (49)		8.0 (12)		81.5 (110)	
	L	0	90.5 (19)*		32.0 (8)*		100.0 (24)*	
- FUSED ^b	F	0	0.8 (1)		0		8.9 (12)	
	L	0	4.8 (1)		0		33.3 (8)*	
- EXTRA ^{+b}	F	0	0.8 (1)		0.7 (1)		1.5 (2)	
	L	0	4.8 (1)		4.0 (1)		8.3 (2)	
- MISSING ^{+b}	F	0	0		0		1.5 (2)	
	L	0	0		0		8.3 (2)	
- IRREG PATTERN OSSIFICATION ^b	F	0	0		0.7 (1)		1.5 (2)	
	L	0	0		4.0 (1)		8.3 (2)	

Table 17 (continued)

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Incidence of Fetal Alterations Summary-Part II

MG/KG/DAY	0		GA 650		NaG 833		EG 2500	
	EXTERNAL EXAMINATION	NUMBER OF FETUSES (NUMBER OF LITTERS)	NUMBER OF FETUSES (NUMBER OF LITTERS)	EXAMINED	EXAMINED	EXAMINED	EXAMINED	EXAMINED
- FUSED ^{+b}	F	0	12.5 (16)	0	62.4 (83)			
	L	0	42.9 (9)*	0	95.8 (23)*			
- D.O. ^b	F	2.0 (3)	10.2 (13)	8.6 (13)	26.3 (35)			
	L	12.5 (3)	38.1 (8)*	32.0 (8)*	83.3 (20)*			
- CLASS I WAVY ^b	F	2.0 (3)	0	8.6 (13)	3.0 (4)			
	L	12.5(3)	0	32.0 (8)	12.5 (3)			
- CLASS II WAVY ^{+b}	F	0	0	4.6 (7)	3.0 (4)			
	L	0	0	20.0 (5)	12.5 (3)			
- EXTRA ^{+b}	F	0	3.1(4)	0	4.5 (6)			
	L	0	9.5(2)	0	20.8 (5)			
- FLOATING ^b	F	0	12.5 (15)	0	48.9 (65)			
	L	0	33.3 (7)*	0	91.7 (22)*			
- MISSING ^{+b}	F	0	23.4 (30)	0.7 (1)	60.2 (80)			
	L	0	71.4 (15)*	4.0 (1)	91.7 (22)*			
- CALLOUSED ^b	F	0.7 (1)	0	2.6 (4)	0.8 (1)			
	L	4.2 (1)	0	12.0(3)	4.2 (1)			
- FORKED ^{+b}	F	0	0	0	1.5 (2)			
	L	0	0	0	8.3 (2)			

PERCENT AFFECTED (NUMBER AFFECTED)

Table 17 (continued)

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Incidence of Fetal Alterations Summary-Part II

MG/KG/DAY	0	GA 650	NaG 833	EG 2500
EXTERNAL EXAMINATION	NUMBER OF FETUSES (NUMBER OF LITERS) EXAMINED			
VISCERAL EXAMINATION	313 (24)	264 (21)	314 (25)	286 (24)
SKELETAL EXAMINATION	161 (24)	137 (21)	163 (25)	151 (24)
	152 (24)	128 (21)	151 (25)	135 (24)
		PERCENT AFFECTED (NUMBER AFFECTED)		
STERNEBRAE - D.O. ^b	F 15.8 (24)	46.0 (58)	6.6 (10)	75.0 (99)
	L 58.3 (14)	85.7 (18)*	32.0 (8)	100.0 (24)*
- FUSED ^b	F 0	1.6 (2)	0	6.8 (9)
	L 0	9.5 (2)	0	33.3 (8)*
- IRREGULAR PATTERN OF OSSIFICATION ^b	F 0.7 (1)	41.3 (52)	5.3 (8)	54.5 (72)
	L 4.2 (1)	90.5 (19)*	28.0 (7)*	91.7 (22)*
- EXTRA SITE OF OSSIFICATION ^b	F 0	2.4 (3)	0.7 (1)	1.5 (2)
	L 0	14.3 (3)	4.0 (1)	8.3 (2)*
LIMBS				
- BENT RADIUS, ULNA, TIBIA, FIBULA ^a	F 0	0	0	0.7 (1)
	L 0	0	0	4.2 (1)
- METACARPALS- D.O.	F 0	0	0	1.5 (2)
	L 0	0	0	8.3 (2)
TOTAL MALFORMED	F 0.3 (1)	23.5 (62)	3.8 (12)	58.0 (166)
	L 4.2 (1)	81.0 (17)*	36.0 (9)*	100.0 (24)*

* SIGNIFICANTLY GREATER INCIDENCE THAN CONTROL (P<0.05)

+ CONSIDERED A MALFORMATION; D.O. = DELAYED OSSIFICATION

a F=FETUSES; L=LITTERS

b BASE NUMBER OF FETUSES ADJUSTED TO ACCOUNT FOR BONES DAMAGED DURING SKELETAL PROCESSING (COULD NOT BE EVALUATED).

Table 18

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Comparison Of Significant Fetal Alterations-Part II

Alteration	2500 EG	650 GA	833 NaG
Decreased fetal body weight	X	X	X
<i>External Malformations</i>			
Meningoencephalocele	X		
Exencephaly	X		
Cleft Lip	X		
Cleft Palate	X		
Omphalocele/gastroschisis/umbilical hernia (& assoc. Limb rotations)	X	S	
<i>Visceral Malformations</i>			
Dilated cerebral ventricles	X	S	
Anophthalmia	T		
Microphthalmia	T		
<i>Skeletal Variations</i>			
D.O. cervical centra	X	X	X
D.O. thoracic centra	X	X	X
D.O. lumbar centra	X	X	X
D.O. sternebra	X	X	X
Fused sternebrae	X	S	
Irreg. pattern sternebrae ossification	X	X	X
Skull-D.O.	X	S	

Table 18 (continued)

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Comparison Of Significant Fetal Alterations-Part II

Alteration	2500 EG	650 GA	833 NaC
<i>Skeletal Variations (continued)</i>			
Skull-extra site of ossification	X	S	X
D.O. ribs	X	X	
Floating ribs	X	X	
D.O. lumbar vertebrae	X		
<i>Skeletal malformations</i>			
Missing vertebrae	X	X	S
Hemivertebrae	X	X	
Fused vertebrae	X	S	
Extra vertebrae	X	S	
Fused centra	X	S	
Missing ribs	X	X	S
Fused ribs	X	X	

X=Statistically significant difference from control

T=Trend, but not statistically identified

S=Similar to EG, but not statistically identified.

Table A- 1

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Individual Acid-Base Values-Part I

RAT ID	GROUP	BLOOD pH					2 1/2h
		0h	1h	3h	6h	9h	
95A8122	CONTROL	7.518	7.461	7.512	7.507	***	***
95A8123	CONTROL	7.494	7.482	7.468	7.471	7.526	7.498
95A8124	CONTROL	7.566	7.482	7.464	7.489	7.578	***
95A8125	CONTROL	7.489	7.476	7.500	7.477	B	B
95A8126	CONTROL	7.501	7.572	7.495	7.571	7.528	7.530
MEAN:		7.514	7.495	7.488	7.503	7.544	7.514
SD:		0.031	0.044	0.021	0.040	0.029	0.023
N:		5	5	5	5	3	2
95A8127	GA 650	7.510	7.457	7.423	7.438	B	B
95A8128	GA 650	7.531	7.463	7.421	7.438	7.440	7.471
95A8129	GA 650	7.506	7.461	7.446	7.451	7.486	B
95A8130	GA 650	7.484	7.391	7.323	7.374	7.436	7.465
95A8131	GA 650	7.524	7.415	7.429	7.410	7.440	7.552
MEAN:		7.511	7.437	7.408	7.422	7.451	7.496
SD:		0.018	0.033	0.049	0.031	0.024	0.049
N:		5	5	5	5	4	3

*** DEAD ANIMAL

B --CANNULA NOT PATENT, UNABLE TO OBTAIN BLOOD SAMPLE.

Table A- 1 (continued)

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Individual Acid-Base Values-Part I

RAT ID	GROUP	BLOOD pH					
		0 h	1h	3h	6h	9h	24h
95B0536	NaG 833	7.4930	7.5210	7.5230	7.5120	7.5250	7.4680
95A8133	NaG 833	7.516	7.536	7.506	7.509	***	***
95A8134	NaG 833	7.498	7.540	7.537	7.553	7.545	7.528
95A8135	NaG 833	7.489	7.506	7.509	7.508	7.533	7.440
95A8136	NaG 833	7.512	7.549	7.535	7.571	7.559	7.526
	MEAN:	7.504	7.533	7.522	7.535	7.546	7.498
	SD:	0.013	0.019	0.017	0.032	0.013	0.050
	N:	4	4	4	4	3	3
95A8137	EG 2500	7.539	7.359	7.396	7.399	7.427	7.472
95A8138	EG 2500	7.488	7.462	7.440	7.461	7.465	7.476
95A8139	EG 2500	7.523	7.456	7.407	7.422	7.441	7.525
95A8140	EG 2500	7.459	7.394	7.315	7.264	7.311	7.483
95A8141	EG 2500	7.485	7.448	7.405	7.386	7.421	7.543
	MEAN:	7.499	7.424	7.393	7.386	7.413	7.500
	SD:	0.032	0.045	0.046	0.074	0.059	0.032
	N:	5	5	5	5	5	5

*** DEAD ANIMAL
 0 Non-pregnant animals excluded

Table A-1 (continued)

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Individual Acid-Base Values-Part I

RAT ID	GROUP	ARTERIAL PCO2 (mmHg)					
		0h	1h	3h	6h	9h	24h
95A8122	CONTROL	28.3	38.0	31.6	30.2	***	***
95A8123	CONTROL	36.6	41.1	39.1	36.9	36.6	40.9
95A8124	CONTROL	30.7	38.3	41.6	38.9	29.1	***
95A8125	CONTROL	37.3	36.6	36.8	37.4	B	B
95A8126	CONTROL	35.7	28.6	33.9	27.9	30.5	31.3
	MEAN:	33.7	36.5	36.6	34.3	32.1	36.1
	SD:	3.99	4.72	3.99	4.88	3.99	6.79
	N:	5	5	5	5	3	2
95A8127	GA 650	33.5	32.2	32.4	34.5	B	B
95A8128	GA 650	31.7	30.1	33.5	32.4	36.0	---a
95A8129	GA 650	34.0	30.0	28.8	31.7	31.2	B
95A8130	GA 650	37.0	35.6	41.3	37.6	34.5	39.4
95A8131	GA 650	29.0	32.6	24.9	30.1	30.1	28.3
	MEAN:	33.0	32.1	32.2	33.3	33.0	33.9
	SD:	2.96	2.29	6.11	2.90	2.76	7.85
	N:	5	5	5	5	4	2

*** DEAD ANIMAL

B = CANNULA NOT PATENT, UNABLE TO OBTAIN BLOOD SAMPLE

---a=SAMPLE ONLY SAMPLED FOR PH DUE TO BLOOD GAS PROGRAMMING ERROR

Table A-1 (continued)

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Individual Acid-Base Values-Part I

RAT ID	GROUP	ARTERIAL PCO ₂ (mmHg)					
		0h	1h	3h	6h	9h	24h
95B0536	NaG 833	36.40	31.90	35.30	35.70	34.70	35.50
95A8133	NaG 833	34.6	36.8	40.4	38.7	***	***
95A8134	NaG 833	34.0	31.9	34.7	31.9	33.2	28.8
95A8135	NaG 833	36.5	36.0	37.6	36.0	34.0	43.8
95A8136	NaG 833	33.6	32.1	32.4	34.1	34.7	33.6
	MEAN:	34.7	34.2	36.3	35.2	34.0	35.4
	SD:	1.28	2.56	3.48	2.89	0.75	7.66
	N:	4	4	4	4	3	3
95A8137	BG 2500	38.3	54.8	28.2	34.2	34.2	44.5
95A8138	BG 2500	34.8	31.4	27.0	29.8	31.5	34.4
95A8139	BG 2500	36.3	35.0	33.1	29.8	30.3	34.5
95A8140	BG 2500	37.1	38.8	36.4	30.8	26.2	32.4
95A8141	BG 2500	34.6	31.3	27.1	28.0	27.7	30.7
	MEAN:	36.2	38.3	30.4	30.5	30.0	35.3
	SD:	1.56	9.75	4.20	2.29	3.15	5.38
	N:	5	5	5	5	5	5

*** DEAD ANIMAL
 0 Non-pregnant animals excluded

Table A-1 (continued)

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Individual Acid-Base Values-Part I

RAT ID	GROUP	BLOOD BICARBONATE (HCO ₃ ; mmol/L)					
		0h	1h	3h	6h	9h	24h
95A8122	CONTROL	23.0	27.0	25.3	23.9	***	***
95A8123	CONTROL	28.1	30.7	28.3	26.9	30.3	31.7
95A8124	CONTROL	27.8	28.6	29.8	29.5	27.1	***
95A8125	CONTROL	28.3	27.0	28.7	27.6	B	B
95A8126	CONTROL	27.9	26.3	26.1	25.6	25.3	26.1
MEAN:		27.0	27.9	27.6	26.7	27.6	28.9
SD:		2.26	1.77	1.88	2.11	2.53	3.96
N:		5	5	5	5	3	2
95A8127	GA 650	26.7	22.7	21.1	23.3	B	B
95A8128	GA 650	26.5	21.5	21.7	21.9	24.0	---a
95A8129	GA 650	26.8	21.4	19.8	22.1	23.5	B
95A8130	GA 650	27.8	21.6	21.4	21.9	23.2	28.3
95A8131	GA 650	23.9	20.9	16.5	19.1	20.4	24.8
MEAN:		26.3	21.6	20.1	21.7	22.8	26.6
SD:		1.45	0.66	2.14	1.55	1.62	2.47
N:		5	5	5	5	4	2

*** DEAD ANIMAL

B = CANNULA NOT PATENT, UNABLE TO OBTAIN BLOOD SAMPLE
 ---a = SAMPLE ONLY SAMPLED FOR PH DUE TO BLOOD GAS
 PROGRAMMING ERROR

Table A-1 (continued)

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Individual Acid-Base Values-Part I

RAT ID	GROUP	BLOOD BICARBONATE (HCO ₃ ; mmol/L)					
		0 h	1h	3h	5h	9h	24h
95B0536	NaG 833	27.90	26.10	29.00	28.60	28.60	25.70
95A8133	NaG 833	28.0	31.1	31.9	30.8	***	***
95A8134	NaG 833	26.4	27.2	29.4	28.1	28.7	23.9
95A8135	NaG 833	27.7	28.4	29.9	28.6	28.6	29.7
95A8136	NaG 833	26.9	28.0	27.3	31.3	31.0	27.8
	MEAN:	27.3	28.7	29.6	29.7	29.4	27.1
	SD:	0.73	1.69	1.89	1.59	1.36	2.96
	N:	4	4	4	4	3	3
95A8137	BG 2500	32.6	30.8	17.3	21.1	22.5	32.5
95A8138	BG 2500	26.4	22.4	18.3	21.2	22.6	25.3
95A8139	BG 2500	29.8	24.6	20.8	19.4	20.6	28.5
95A8140	BG 2500	26.3	23.7	18.5	13.9	13.2	24.3
95A8141	BG 2500	26.0	21.6	17.0	16.8	18.0	26.4
	MEAN:	28.2	24.6	18.4	18.5	19.4	27.4
	SD:	2.90	3.64	1.50	3.12	3.93	3.25
	N:	5	5	5	5	5	5

*** DEAD ANIMAL
 0 Non-pregnant animals excluded

Table A- 2

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Individual Serum Glycolate (µg/ml) Values-Part I

<u>RAF I.D.</u>	<u>GROUP</u>	<u>0 h</u>	<u>1 h</u>	<u>3 h</u>	<u>6 h</u>	<u>9 h</u>	<u>24 h</u>
95A8122	CONTROL	N.S. (b)	N.S. (c)	N.D.	N.D.	N.S. (a)	N.S. (a)
95A8123	CONTROL	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
95A8124	CONTROL	N.D.	N.D.	N.D.	N.D.	N.D.	N.S. (a)
95A8125	CONTROL	N.D.	N.D.	N.D.	N.D.	N.S. (a)	N.S. (a)
95A8126	CONTROL	N.S. (a)	N.D.	N.D.	N.D.	N.D.	N.D.
95A8127	GA 650	N.S. (b)	216	574	N.S. (c)	N.S. (a)	N.S. (a)
95A8128	GA 650	N.S. (a)	N.S. (c)	431	392	17	N.D.
95A8129	GA 650	N.D. (d)	382	661	261	112	N.S. (a)
95A8130	GA 650	N.D. (d)	343	672	331	129	N.D.
95A8131	GA 650	N.S. (a)	413	841	629	374	N.D.

N.D. = Not Detected
 N.D. (d) = Not Detected; slightly less than 25 µl sample taken for analysis.
 N.S. (a) = No Sample; not submitted for this sampling point.
 N.S. (b) = No Sample; not enough sample left to sample.
 N.S. (c) = No Sample; not sampled as sample appeared gelatinous and not homogeneous.

Table A-2 (continued)

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Individual Serum Glycolate (µg/ml) Values-Part I

RAT I.D.	GROUP	Values (µg/ml)							
		0 h	1 h	3 h	6 h	9 h	24 h		
95A8133	NaG 833	N.S. (a)	573	329	145	N.S. (a)	N.S. (a)		
95A8134	NaG 833	N.D.	722	529	299	N.D.	N.D.		
95A8135	NaG 833	N.D.	594	341	22	N.D.	N.D.		
95A8136	NaG 833	N.S. (a)	724	495	263	N.D.	N.D.		
95B0536	NaG 833	N.S. (b)	648	440	278	N.D.	N.D.		
95A8137	EG 2500	N.S. (b)	294	608	555	N.S. (b)	N.D.		
95A8138	EG 2500	N.S. (b)	584	664	545	142	N.D.		
95A8139	EG 2500	N.S. (b)	433	708	710	790	N.D.		
95A8140	EG 2500	N.D.	368	680	608	498	N.D.		
95A8141	EG 2500	N.D.	370	666	575	333	N.D.		

N.D. = Not Detected
 N.D. (d) = Not Detected; slightly less than 25 µl sample taken for analysis.
 N.S. (a) = No Sample; not submitted for this sampling point.
 N.S. (b) = No Sample; not enough sample left to sample.
 N.S. (c) = No Sample; not sampled as sample appeared gelatinous and not homogeneous.

Table A- 3

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Individual In-Life Observations-Part I

<u>RAT ID</u>	<u>GROUP</u>	<u>TEST DAY</u>	<u>OBSERVATIONS</u>
95A8122	CONTROL	GD 10	CONVULSIONS
		GD 10	SACRIFICED
95A8123	CONTROL	GD 10	NO REMARKABLE OBSERVATIONS
		GD 11	SACRIFICED
95A8124	CONTROL	GD 10	NO REMARKABLE OBSERVATIONS
		GD 11	DECREASED ACTIVITY
		GD 11	FOUND DEAD
95A8125	CONTROL	GD 10	NO REMARKABLE OBSERVATIONS
		GD 11	SACRIFICED
95A8126	CONTROL	GD 10	NO REMARKABLE OBSERVATIONS
		GD 11	SACRIFICED
95A8127	GA 650	GD 10	NO REMARKABLE OBSERVATIONS
		GD 11	SACRIFICED
95A8128	GA 650	GD 10	NO REMARKABLE OBSERVATIONS
		GD 11	SACRIFICED
95A8129	GA 650	GD 10	NO REMARKABLE OBSERVATIONS
		GD 11	SACRIFICED
95A8130	GA 650	GD 10	NO REMARKABLE OBSERVATIONS
		GD 11	SACRIFICED
95A8131	GA 650	GD 10	NO REMARKABLE OBSERVATIONS
		GD 11	SACRIFICED

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Table A- 3 (continued)

Individual In-Life Observations-Part I

<u>RAW ID</u>	<u>GROUP</u>	<u>TEST DAY</u>	<u>OBSERVATIONS</u>
95B0536	NaG 833	GD 10	NO REMARKABLE OBSERVATIONS
		GD 11	SACRIFICED
95A8133	NaG 833	GD 10	FOUND LYING ON SIDE IN MORIBUND CONDITION
		GD 10	SACRIFICED
95A8134	NaG 833	GD 10	NO REMARKABLE OBSERVATIONS
		GD 11	SACRIFICED
95A8135	NaG 833	GD 10	NO REMARKABLE OBSERVATIONS
		GD 11	SACRIFICED
95A8136	NaG 833	GD 10	NO REMARKABLE OBSERVATIONS
		GD 11	SACRIFICED
95A8137	EG 2500	GD 10	NO REMARKABLE OBSERVATIONS
		GD 11	SACRIFICED
95A8138	EG 2500	GD 10	NO REMARKABLE OBSERVATIONS
		GD 11	SACRIFICED
95A8139	EG 2500	GD 10	NO REMARKABLE OBSERVATIONS
		GD 11	SACRIFICED
95A8140	EG 2500	GD 10	NO REMARKABLE OBSERVATIONS
		GD 11	SACRIFICED
95A8141	EG 2500	GD 10	NO REMARKABLE OBSERVATIONS
		GD 11	SACRIFICED

Table A- 4

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Individual Gestation Body Weights-Part I

RAT ID	GROUP	PRE-SURG		TERMINAL	BW GAIN	
		GD 8 BW (GRAMS)	GD10 BW (GRAMS)	BW (GRAMS)	GD 8 -10 (GRAMS)	BW GAIN GD 10-TERM (GRAMS)
95A8122	CONTROL	277	239	*****	-38	*****
95A8123	CONTROL	309	300	302	-9	2
95A8124	CONTROL	285	260	*****	-25	*****
95A8125	CONTROL	238	207	207	-31	0
95A8126	CONTROL	290	271	273	-19	2
MEAN:		279.8	255.4	260.7	-24.4	-1.3
SD:		26.2	34.9	48.9	11.1	1.2
N:		5	5	3	5	3
95A8127	GA 650	275	264	250	-11	-14
95A8128	GA 650	294	277	255	-17	-22
95A8129	GA 650	243	246	247	3	1
95A8130	GA 650	233	204	200	-29	-4
95A8131	GA 650	320	282	273	-38	-9
MEAN:		273.0	254.6	245.0	-18.4	-9.6
SD:		35.9	31.5	27.1	15.9	8.9
N:		5	5	5	5	5

***** DEAD ANIMAL,
 BLOCKED CANNULA ANIMALS INCLUDED IN CALCULATION OF MEANS AND STANDARD
 DEVIATIONS

Table A- 4 (continued)

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Individual Gestation Body Weights-Part I

RAT ID	GROUP	PRE-SURG		TERMINAL	BW GAIN	
		GD 8 BW	GD10 BW	BW	GD 8 -10	GD 10-TERM
95B0536	NaG 833	3020	2930	2860	-90	-70
95A8133	NaG 833	287	272	*****	-15	*****
95A8134	NaG 833	290	266	260	-24	-6
95A8135	NaG 833	230	226	222	-4	-4
95A8136	NaG 833	326	294	290	-32	-4
MEAN:		283.3	264.5	257.3	-18.8	-4.7
SD:		39.7	28.3	34.1	12.0	1.2
N:		4	4	3	4	3
95A8137	BG 2500	273	246	228	-27	-18
95A8138	BG 2500	220	222	223	2	1
95A8139	BG 2500	215	184	184	-31	0
95A8140	BG 2500	234	200	185	-34	-15
95A8141	BG 2500	296	281	283	-15	2
MEAN:		247.6	226.6	220.6	-21.0	-6.0
SD:		35.3	38.3	40.5	14.7	9.7
N:		5	5	5	5	5

***** DEAD ANIMAL
 0 NON-PREGNANT ANIMALS EXCLUDED FROM CALCULATION OF MEANS AND STANDARD DEVIATIONS
 BLOCKED CANNULA ANIMALS INCLUDED IN CALCULATION OF MEANS AND STANDARD DEVIATIONS

Table A- 5

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYOXALATE ANION AND METABOLIC ACIDOSIS

Necropsy Data-Part I

RAT ID	GROUP	Survived		DIED/ SAC'D	Cannula		PREGNANCY STATUS	POST-MORTEM FINDINGS
		to scheduled necropsy?	SAC.		placed OK?	PREGNANT		
95A8122	CONTROL	NO	NO	YES	PREGNANT	NVL		
95A8123	CONTROL	YES	NO	YES	PREGNANT	NVL		
95A8124	CONTROL	NO	DEAD	YES	PREGNANT	clotted blood under skin ventral incision site		
95A8125	CONTROL	YES	NO	YES	PREGNANT	clot found in carotid proximal to cannula tip		
95A8126	CONTROL	YES	NO	YES	PREGNANT	NVL		
95A8127	GA 650	YES	NO	YES	PREGNANT	cannula contains clotted blood		
95A8128	GA 650	YES	NO	YES	PREGNANT	NVL		
95A8129	GA 650	YES	NO	YES	PREGNANT	clot at tip of cannula		
95A8130	GA 650	YES	NO	YES	PREGNANT	NVL		
95A8131	GA 650	YES	NO	YES	PREGNANT	NVL		

NVL=NO VISIBLE LESIONS

Table A- 5 (continued)
 ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Necropsy Data-Part I

RAT ID	GROUP	Survived		DIED/ SAC'D	Cannula		PREGNANCY STATUS	POST-MORTEM FINDINGS
		to scheduled necropsy?	placed OK?		NOT PREG.	clotted blood under ventral incision site		
95B0536	NaG 833	YES	NO	NO	YES	PREGNANT	NVL	
95A8133	NaG 833	NO	NO	SAC.	YES	PREGNANT	NVL	
95A8134	NaG 833	YES	NO	NO	YES	PREGNANT	NVL	
95A8135	NaG 833	YES	NO	NO	YES	PREGNANT	NVL	
95A8136	NaG 833	YES	NO	NO	YES	PREGNANT	NVL	
95A8137	EG 2500	YES	NO	NO	YES	PREGNANT	NVL	
95A8138	EG 2500	YES	NO	NO	YES	PREGNANT	NVL	
95A8139	EG 2500	YES	NO	NO	YES	PREGNANT	NVL	
95A8140	EG 2500	YES	NO	NO	YES	PREGNANT	NVL	
95A8141	EG 2500	YES	NO	NO	YES	PREGNANT	NVL	

NVL=NO VISIBLE LESIONS

Table A- 6

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Individual In-Life Observations-Part II

DOSE MG/KG/DAY	ANIMAL NUMBER	TEST DAY	EXAM TYPE#	OBSERVATION/COMMENT
0	96A0832	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED
	96A0833	G 0 - G 9 G 10 - G 21 G 21	CL CL	NO REMARKABLE OBSERVATIONS MASS/NODULE, WART-LIKE, FIRM, < 0.5 CM, TAIL, MIDLINE SACRIFICED
	96A0834	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED
	96A0835	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED
	96A0836	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED
	96A0837	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED
	96A0838	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED
	96A0839	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED
	96A0840	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED
	96A0841	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED
	96A0842	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED

EXAM TYPES: CG = CAGESIDE, CL = CLINICAL, CG* = CAGESIDE, NOTED ON CLINICAL EXAM, CL* = CLINICAL, NOTED ON CAGESIDE EXAM.

Table A-6 (continued)

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Individual In-Life Observations-Part II

DOSE MG/KG/DAY	ANIMAL NUMBER	TEST DAY	EXAM TYPE#	OBSERVATION/COMMENT
0	96A0843	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED
	96A0844	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED
	96A0845	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED
	96A0846	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED
	96A0847	G 0 - G 7 G 8 - G 21 G 21	CL CL	NO REMARKABLE OBSERVATIONS MASS/NODULE, RIGHT, HIP, FIRM, 2.6 - 5.0 CM SACRIFICED
	96A0848	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED
	96A0849	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED
	96A0850	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED
	96A0851	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED
	96A0852	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED
	96A0853	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED

EXAM TYPES: CG = CAGESIDE, CL = CLINICAL, CG* = CAGESIDE, NOTED ON CLINICAL EXAM, CL* = CLINICAL, NOTED ON CAGESIDE EXAM.

Table A-6 (continued)

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

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Individual In-Life Observations-Part II

DOSE MG/KG/DAY	ANIMAL NUMBER	TEST DAY	EXAM TYPE#	OBSERVATION/COMMENT
0	96A0854	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED
	96A0855	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED
	96A0856	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED
650	96A0857	G 0 - G 13 G 14 - G 14 G 14 - G 14	CL	NO REMARKABLE OBSERVATIONS RESPIRATION NOISY RESPIRATION DEEP FACIAL SOILING MOUTH BREATHING SALIVATION MORIBUND
	96A0858	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED
	96A0859	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED
	96A0860	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED
	96A0861	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED
	96A0862	G 0 - G 7 G 8	CL	NO REMARKABLE OBSERVATIONS SPONTANEOUS DEATH
	96A0863	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED

* EXAM TYPES: CG = CAGESIDE, CL = CLINICAL, CG* = CAGESIDE, NOTED ON CLINICAL EXAM, CL* = CLINICAL, NOTED ON CAGESIDE EXAM.

Table A- 6 (continued)

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Individual In-Life Observations-Part II

DOSE MG/KG/DAY	ANIMAL NUMBER	TEST DAY	EXAM TYPE#	OBSERVATION/COMMENT
650	96A0864	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED
	96A0865	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED
	96A0866	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED
	96A0867	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED
	96A0868	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED
	96A0869	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED
	96A0870	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED
	96A0871	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED
	96A0872	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED
	96A0873	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED
	96A0874	G 0 - G 6 G 7	CL	NO REMARKABLE OBSERVATIONS MORIBUND
	96A0875	G 0 - G 20	CL	NO REMARKABLE OBSERVATIONS

EXAM TYPES: CG = CAGESIDE, CL = CLINICAL, CG* = CAGESIDE, NOTED ON CLINICAL EXAM, CL* = CLINICAL, NOTED ON CAGESIDE EXAM.

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Table A- 6 (continued)

Individual In-Life Observations-Part II

DOSE MG/KG/DAY	ANIMAL NUMBER	TEST DAY	EXAM TYPE#	OBSERVATION/COMMENT
650	96A0875	G 21	CL	SACRIFICED
	96A0876	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED
	96A0877	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED
650	96A0878	G 0 - G 12	CL	NO REMARKABLE OBSERVATIONS
		G 13 - G 14	CL	RESPIRATION NOISY
		G 13 - G 14	CL	SALIVATION
		G 14 - G 14	CL	EXCESSIVE CHROMORRHORRHEA
		G 14 - G 14	CL	MOUTH BREATHING MORIBUND
96A0879	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED	
96A0880	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED	
96A0881	96A0881	G 0 - G 6	CL	NO REMARKABLE OBSERVATIONS
		G 7 - G 8	CL	RESPIRATION NOISY
		G 7 - G 8	CL	EXCESSIVE CHROMORRHORRHEA
		G 7 - G 8	CL	EXCESSIVE CHROMODACRYORRHEA,
		G 9 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED
833	96A0882	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED
		96A0883	G 0 - G 20 G 21	CL

EXAM TYPES: CG = CAGESIDE, CL = CLINICAL, CG* = CAGESIDE, NOTED ON CLINICAL EXAM, CL* = CLINICAL, NOTED ON CAGESIDE EXAM.

Table A- 6 (continued)

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Individual In-Life Observations-Part II

DOSE MG/KG/DAY	ANIMAL NUMBER	TEST DAY	EXAM TYPE#	OBSERVATION/COMMENT
833	96A0884	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED
	96A0885	G 0 - G 10 G 11 - G 21 G 21	CL CL	NO REMARKABLE OBSERVATIONS ABRASION, SCABBED, LEFT, HIP SACRIFICED
	96A0886	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED
	96A0887	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED
	96A0888	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED
	96A0889	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED
	96A0890	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED
	96A0891	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED
	96A0892	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED
	96A0893	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED
	96A0894	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED

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Table A- 6 (continued)

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Individual In-Life Observations-Part II

DOSE MG/KG/DAY	ANIMAL NUMBER	TEST DAY	EXAM TYPE#	OBSERVATION/COMMENT
833	96A0895	0 - G 20	CL	NO REMARKABLE OBSERVATIONS
		G 21		SACRIFICED
	96A0896	0 - G 20	CL	NO REMARKABLE OBSERVATIONS
		G 21		SACRIFICED
	96A0897	0 - G 20	CL	NO REMARKABLE OBSERVATIONS
		G 21		SACRIFICED
	96A0898	0 - G 20	CL	NO REMARKABLE OBSERVATIONS
		G 21		SACRIFICED
	96A0899	0 - G 20	CL	NO REMARKABLE OBSERVATIONS
		G 21		SACRIFICED
	96A0900	0 - G 20	CL	NO REMARKABLE OBSERVATIONS
		G 21		SACRIFICED
	96A0901	0 - G 20	CL	NO REMARKABLE OBSERVATIONS
		G 21		SACRIFICED
	96A0902	0 - G 20	CL	NO REMARKABLE OBSERVATIONS
		G 21		SACRIFICED
	96A0903	0 - G 20	CL	NO REMARKABLE OBSERVATIONS
		G 21		SACRIFICED
	96A0904	0 - G 20	CL	NO REMARKABLE OBSERVATIONS
		G 21		SACRIFICED
	96A0905	0 - G 20	CL	NO REMARKABLE OBSERVATIONS
		G 21		SACRIFICED
	96A0906	0 - G 20	CL	NO REMARKABLE OBSERVATIONS
		G 21		SACRIFICED

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Table A- 6 (continued)

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Individual In-Life Observations-Part II

DOSE MG/KG/DAY	ANIMAL NUMBER	TEST DAY	EXAM TYPE#	OBSERVATION/COMMENT
833	96A0906	G 21	CL	SACRIFICED
2500	96A0907	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED
	96A0908	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED
	96A0909	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED
	96A0910	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED
	96A0911	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED
	96A0912	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED
	96A0913	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED
	96A0914	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED
	96A0915	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED
	96A0916	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED
	96A0917	G 0 - G 20 G 21	CL	NO REMARKABLE OBSERVATIONS SACRIFICED

* EXAM TYPES: CG = CAGESIDE, CL = CLINICAL, CG* = CAGESIDE, NOTED ON CLINICAL EXAM, CL* = CLINICAL, NOTED ON CAGESIDE EXAM.

Table A- 6 (continued)

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Individual In-Life Observations-Part II

DOSE MG/KG/DAY	ANIMAL NUMBER	TEST DAY	EXAM TYPE#	OBSERVATION/COMMENT
2500	96A0918	G 0 - G 20	CL	NO REMARKABLE OBSERVATIONS
		G 21		SACRIFICED
	96A0919	G 0 - G 20	CL	NO REMARKABLE OBSERVATIONS
		G 21		SACRIFICED
	96A0920	G 0 - G 20	CL	NO REMARKABLE OBSERVATIONS
		G 21		SACRIFICED
	96A0921	G 0 - G 20	CL	NO REMARKABLE OBSERVATIONS
		G 21		SACRIFICED
	96A0922	G 0 - G 20	CL	NO REMARKABLE OBSERVATIONS
		G 21		SACRIFICED
	96A0923	G 0 - G 18	CL	NO REMARKABLE OBSERVATIONS
		G 19 - G 20	CL	VAGINAL BLEEDING
	96A0924	G 0 - G 20	CL	NO REMARKABLE OBSERVATIONS
		G 21		SACRIFICED
	96A0925	G 0 - G 20	CL	NO REMARKABLE OBSERVATIONS
		G 21		SACRIFICED
	96A0926	G 0 - G 20	CL	NO REMARKABLE OBSERVATIONS
		G 21		SACRIFICED
	96A0927	G 0 - G 20	CL	NO REMARKABLE OBSERVATIONS
		G 21		SACRIFICED
	96A0928	G 0 - G 20	CL	NO REMARKABLE OBSERVATIONS
		G 21		SACRIFICED

EXAM TYPES: CG = CAGESIDE, CL = CLINICAL, CG* = CAGESIDE, NOTED ON CLINICAL EXAM, CL* = CLINICAL, NOTED ON CAGESIDE EXAM.

Table A- 6 (continued)

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Individual In-Life Observations-Part II

DOSE MG/KG/DAY	ANIMAL NUMBER	TEST DAY	EXAM TYPE#	OBSERVATION/COMMENT
2500	96A0929	G 0 - G 20	CL	NO REMARKABLE OBSERVATIONS
		G 21		SACRIFICED
	96A0930	G 0 - G 20	CL	NO REMARKABLE OBSERVATIONS
		G 21		SACRIFICED
	96A0931	G 0 - G 20	CL	NO REMARKABLE OBSERVATIONS
		G 21		SACRIFICED

EXAM TYPES: CG = CAGESIDE, CL = CLINICAL, CG* = CAGESIDE, NOTED ON CLINICAL EXAM, CL* = CLINICAL, NOTED ON CAGESIDE EXAM.

Table A- 7

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Individual Feed Consumption (G) -Part II

DOSE MG/KG/DAY	ANIMAL NUMBER	DAYS OF GESTATION									
		3-6	6-9	9-12	12-16	16-19	19-21				
0	96A0832	20.3	20.6	21.6	24.8	27.5	26.4				
	96A0833	19.5	21.5	23.3	24.8	28.0	26.5				
	96A0834	24.3	22.2	23.6	23.5	25.4	24.6				
	96A0835	24.2	24.2	24.6	27.3	29.2	27.0				
	96A0836	23.8	24.3	24.5	26.2	28.9	25.4				
	96A0837	19.4 ⁰	20.7 ⁰	21.0 ⁰	16.8 ⁰	16.2 ⁰	18.9 ⁰				
	96A0838	24.3	24.7	27.6	26.4	29.9	26.2				
	96A0839	24.9	24.3	24.6	27.2	28.9	26.6				
	96A0840	17.5	20.7	24.1	25.4	24.8	23.3				
	96A0841	20.3	22.6	23.7	26.6	29.3	25.6				
	96A0842	21.7	24.1	24.6	27.2	29.2	26.1				
	96A0843	20.7	22.6	26.4	27.3	29.4	26.1				
	96A0844	20.5	21.7	25.3	24.8	26.6	25.3				
	96A0845	17.5	17.2	18.5	21.7	26.8	27.2				
	96A0846	21.1	21.1	24.7	26.7	29.5	27.3				
	96A0847	19.8	20.1	20.8	22.1	25.0	25.7				
	96A0848	17.7	21.2	23.7	24.5	29.7	25.1				
	96A0849	20.1	20.9	22.8	26.6	28.0	27.5				
	96A0850	21.3	22.8	25.4	25.1	27.0	26.2				
	96A0851	22.7	24.3	25.9	26.5	28.4	23.3				
	96A0852	13.9	23.1	23.5	24.8	27.5	27.1				
	96A0853	23.0	23.9	27.3	28.6	30.5	28.9				
	96A0854	24.5	27.6	29.6	28.4	28.5	27.1				
	96A0855	23.1	24.0	26.0	26.8	26.5	25.5				
	96A0856	22.2	21.9	20.9	19.5 [#]	21.3 [#]	21.7				
	MEAN	21.2	22.6	24.3	25.5	27.7	25.9				
	S.D.	2.7	2.1	2.4	2.2	2.1	1.5				
	N=	24	24	24	24	24	24				

0 VALUES EXCLUDED FROM ANALYSIS.
 # STATISTICAL OUTLIERS INCLUDED IN ANALYSIS.
 * ANIMALS WITHOUT VIABLE FETUSES ON DAY 21 WERE EXCLUDED FROM CALCULATION
 OF MEANS AND STANDARD DEVIATIONS

Table A- 7 (continued)

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Individual Feed Consumption (G) -Part II

DOSE MG/KG/DAY	ANIMAL NUMBER	DAYS OF GESTATION									
		3-6	6-9	9-12	12-16	16-19	19-21				
650	96A0857	21.8	23.2	22.9	***	27.0	***	27.2			
	96A0858	22.3	24.1	22.9	25.6	27.0	27.2				
	96A0859	21.7	23.3	22.9	22.9	26.1	23.3				
	96A0860	19.8	21.0	22.2	20.9	23.7	26.7				
	96A0861	19.0	20.9	18.8	18.3	22.6	23.9				
	96A0862	22.2	***	***	***	***	***				
	96A0863	22.3	22.2	22.0	22.8	24.4	22.3				
	96A0864	20.5	22.1	23.6	19.6	23.6	26.2				
	96A0865	22.3	21.8	23.1	20.4	24.2	25.3				
	96A0866	21.8	18.7	2.3#	28.0	30.5	30.4				
	96A0867	20.4	22.6	24.1	24.4	25.7	25.0				
	96A0868	21.0	22.5	24.0	27.2	28.1	25.6				
	96A0869	20.2	22.3	22.4	23.1	24.4	23.5				
	96A0870	20.3	20.9	23.1	21.4	22.3	20.7				
	96A0871	21.8	23.9	25.9	27.3	28.9	24.8				
	96A0872	20.4	20.3	22.5	21.1	22.0	22.7				
	96A0873	22.1	21.4	22.6	22.9	25.3	25.3				
	96A0874	17.3	***	***	***	***	***				
	96A0875	19.2	21.6	20.5	22.2	22.1	20.0				
	96A0876	21.3	25.1	12.7#	28.1	28.8	28.4				
96A0877	22.8	23.0	23.5	23.7	24.9	-169.8*					
96A0878	19.4	20.1	18.5	***	***	***					
96A0879	23.8	22.3	25.4	24.8	29.1	25.6					
96A0880	23.2	23.2	22.8	25.2	27.5	24.3					
96A0881	24.3	10.9#	23.1	21.1	24.5	24.6					
MEAN	21.2	21.6	21.4	23.4	25.5	24.8					
S.D.	1.6	2.7	4.9	2.8	2.5	2.4					
N=	25	23	23	21	21	20					

*** DEAD ANIMAL.
 * EXCLUDED DUE TO ERRONEOUS WEIGHT.
 # STATISTICAL OUTLIERS INCLUDED IN ANALYSIS.
 ANIMALS WITHOUT VIABLE FETUSES ON DAY 21 WERE EXCLUDED FROM CALCULATION
 OF MEANS AND STANDARD DEVIATIONS

Table A- 7 (continued)

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Individual Feed Consumption (g) -Part II

DOSE MG/KG/DAY	ANIMAL NUMBER	DAYS OF GESTATION									
		3-6	6-9	9-12	12-16	16-19	19-21				
833	96A0882	21.4	20.7	21.8	23.1	24.6	23.4				
	96A0883	21.9	20.6	20.8	22.3	25.6	21.6				
	96A0884	20.1	20.5	22.3	25.0	29.5	26.1				
	96A0885	23.4	24.2	24.9	27.2	29.6	27.2				
	96A0886	21.4	20.9	22.6	25.4	26.7	25.3				
	96A0887	22.5	22.4	24.4	26.1	28.9	25.3				
	96A0888	21.5	21.5	25.0	23.3	27.2	26.3				
	96A0889	20.1	19.8	20.6	22.9	24.4	25.1				
	96A0890	20.7	22.6	25.3	28.3	30.7	26.3				
	96A0891	21.7	21.6	22.6	24.8	29.3	27.0				
	96A0892	22.9	21.9	24.7	26.4	29.3	27.7				
	96A0893	21.0	21.3	22.2	24.4	29.0	27.0				
	96A0894	19.0	18.0	20.0	20.3	24.0	21.1				
	96A0895	20.1	20.6	22.2	22.9	26.4	25.3				
	96A0896	17.3	19.8	21.7	23.2	26.9	23.6				
	96A0897	21.2	20.1	23.1	23.3	23.6	22.6				
	96A0898	21.9	19.6	17.7	19.3	23.4	21.5				
	96A0899	21.6	21.3	23.6	25.4	29.6	26.4				
	96A0900	19.2	20.3	23.8	24.7	28.5	25.6				
	96A0901	22.3	23.3	26.2	27.8	32.1	27.8				
	96A0902	20.8	20.8	22.6	24.0	26.0	24.6				
	96A0903	23.9	21.8	24.3	25.0	27.9	26.5				
	96A0904	23.1	22.1	22.8	24.3	27.4	26.7				
	96A0905	23.4	21.2	22.2	24.0	28.3	27.4				
	96A0906	20.3	20.6	21.2	22.6	25.8	25.9				
MEAN		21.3	21.1	22.7	24.2	27.4	25.3				
S.D.		1.5	1.3	1.9	2.1	2.3	2.0				
N=		25	25	25	25	25	25				

ANIMALS WITHOUT VIABLE FETUSES ON DAY 21 WERE EXCLUDED FROM CALCULATION
 OF MEANS AND STANDARD DEVIATIONS

Table A- 7 (continued)

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Individual Feed Consumption (G) -Part II
 DAYS OF GESTATION

DOSE MG/KG/DAY	ANIMAL NUMBER	3-6	6-9	9-12	12-16	16-19	19-21
2500	96A0907	19.2	17.8	20.1	23.4	27.1	23.8
	96A0908	22.2	20.1	19.6	23.0	25.5	23.6
	96A0909	21.6	20.8	20.4	24.1	27.4	25.6
	96A0910	22.1	18.4	20.5	23.9	28.7	25.3
	96A0911	23.4	22.4	23.7	24.4	28.2	25.1
	96A0912	22.5	19.9	22.1	20.3	25.9	27.8
	96A0913	22.2	19.4	21.3	22.9	26.4	21.7
	96A0914	20.4	17.5	16.8	19.5	24.6	24.9
	96A0915	21.6	15.6	19.3	22.4	27.5	23.4
	96A0916	19.5	17.6	18.5	20.5	23.7	20.2
	96A0917	21.2	19.5	20.3	21.9	22.9	20.8
	96A0918	19.5	18.3	20.3	23.1	26.1	24.9
	96A0919	20.3	19.9	20.5	22.7	23.1	24.1
	96A0920	18.5	18.3	19.8	21.4	23.1	22.7
	96A0921	22.2	22.1	25.7	24.6	29.4	26.4
	96A0922	20.5	18.2	18.6	20.7	23.0	20.7
	96A0923	20.4	15.1	17.5	17.2	15.0	17.7
	96A0924	24.5	21.6	21.2	23.3	24.2	25.0
	96A0925	21.5	22.0	24.1	24.7	27.9	27.4
	96A0926	19.8	16.1	18.6	22.7	25.3	26.2
	96A0927	25.6	23.7	24.4	26.0	29.8	27.1
	96A0928	18.1	17.2	19.5	20.2	22.6	22.0
	96A0929	22.9	21.8	21.0	23.2	26.2	26.1
	96A0930	21.2	19.9	21.8	20.9	22.6	23.1
	96A0931	22.7	23.6	23.4	25.8	29.1	28.6
	MEAN	21.4	19.7	20.9	22.7	25.8	24.4
	S.D.	1.8	2.2	2.1	1.8	2.3	2.3
	N=	24	24	24	24	24	24

q VALUES EXCLUDED FROM ANALYSIS.
 ANIMALS WITHOUT VIABLE FETUSES ON DAY 21 WERE EXCLUDED FROM CALCULATION
 OF MEANS AND STANDARD DEVIATIONS

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Table A - 8

Individual Gestation Body Weights (G) -Part II

DOSE MG/KG/DAY	ANIMAL NUMBER	DAY OF GESTATION										
		0	6	9	12	16	21	21(C)				
0	96A0832	233.0	255.7	283.3	300.7	330.4	406.5	294.2				
	96A0833	224.0	255.1	276.7	298.9	322.3	389.8	305.0				
	96A0834	221.0	249.6	266.7	284.8	307.1	379.8	268.8				
	96A0835	215.0	257.8	277.5	298.6	325.1	391.5	288.4				
	96A0836	231.0	269.3	288.5	302.4	341.3	413.8	302.9				
	96A0837	226.0	260.1	271.8	276.7	277.9	280.0					
	96A0838	217.0	268.9	288.7	310.1	344.6	423.8	313.1				
	96A0839	219.0	272.5	287.0	299.0	333.7	411.6	291.0				
	96A0840	205.0	224.4	235.1	254.7	285.9	336.0	253.7				
	96A0841	224.0	249.7	268.8	282.8	322.5	397.8	291.4				
	96A0842	219.0	257.2	279.1	296.4	331.4	411.9	307.1				
	96A0843	227.0	256.5	274.2	299.6	339.9	409.6	312.0				
	96A0844	214.0	245.2	260.5	280.0	304.5	376.8	291.8				
	96A0845	209.0	236.6	250.3	262.6	291.7	366.2	282.5				
	96A0846	227.0	261.8	278.4	290.7	326.4	408.4	306.4				
	96A0847	210.0	234.2	246.5	257.1	286.5	360.3	270.3				
	96A0848	225.0	250.7	262.1	280.7	308.2	386.1	286.8				
	96A0849	216.0	246.2	264.0	282.3	320.8	405.2	306.8				
	96A0850	231.0	262.4	280.5	303.5	332.9	411.0	309.2				
	96A0851	220.0	257.6	277.3	298.1	324.1	383.6	294.5				
	96A0852	210.0	219.7	253.6	274.1	306.2	392.2	291.5				
	96A0853	224.0	255.1	275.4	309.5	340.6	420.7	323.5				
	96A0854	213.0	254.7	282.0	309.0	343.0	423.7	311.2				
	96A0855	220.0	246.7	266.1	298.3	325.6	398.8	300.4				
	96A0856	216.0	238.9	247.1	260.7	284.4	351.2	256.4				
	MEAN	219.6	251.1	269.6	288.9	320.0	394.0	294.1				
	S.D.	7.5	13.3	14.6	16.9	18.9	23.2	17.8				
	N=	24	24	24	24	24	24	24				

--- NO DATA.
 @ VALUES EXCLUDED FROM ANALYSIS.
 ANIMALS WITHOUT VIABLE FETUSES ON DAY 21 WERE EXCLUDED FROM ANALYSIS
 21(C) = DAY 21 BODY WT - GRAVID UTERUS WT

Table A- 8 (continued)

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Individual Gestation Body Weights (G) -Part II
 DAY OF GESTATION

DOSE MG/KG/DAY	ANIMAL NUMBER	0	6	9	12	16	21	21(C)
650	96A0857	230.0	259.2	289.6	310.4	***	388.8	307.3
	96A0858	229.0	257.1	279.9	295.9	319.6	384.2	294.9
	96A0859	216.0	253.7	275.4	299.3	318.3	326.4	313.9
	96A0860	220.0	251.1	270.5	286.4	282.8	343.5	263.7
	96A0861	218.0	241.5	257.6	262.8	260.3	***	***
	96A0862	219.0	256.3	***	***	***	***	***
	96A0863	231.0	266.7	277.8	289.8	320.8	377.9	282.7
	96A0864	224.0	258.9	285.0	305.4	306.1	394.9	302.0
	96A0865	234.0	261.4	274.7	286.7	290.9	370.4	277.7
	96A0866	217.0	245.6	248.0	263.7	305.2	384.9	307.9
	96A0867	224.0	261.4	283.9	294.4	321.7	382.7	305.5
	96A0868	219.0	259.0	275.8	286.7	321.7	385.3	303.4
	96A0869	210.0	240.9	256.1	266.8	286.2	345.3	279.9
	96A0870	219.0	237.0	251.7	263.3	282.8	342.6	270.2
	96A0871	220.0	258.2	278.4	295.9	328.8	412.0	293.3
	96A0872	209.0	240.9	249.9	266.5	294.8	362.1	278.0
	96A0873	226.0	256.2	265.8	288.0	303.0	383.5	287.9
	96A0874	221.0	241.8	241.8	267.5	288.0	332.2	260.4
	96A0875	213.0	240.5	260.1	267.5	288.0	332.2	260.4
	96A0876	233.0	259.5	280.1	259.6	314.9	394.6	300.3
	96A0877	228.0	259.2	269.9	286.2	307.8	369.2	278.8
	96A0878	210.0	231.8	244.2	251.3	***	***	***
	96A0879	217.0	251.8	258.7	280.2	306.9	388.5	288.6
	96A0880	249.0	249.0	256.8	272.8	306.5	378.0	279.2
	96A0881	219.0	246.0	230.9	260.5	277.1	349.9	269.3
MEAN		220.8	251.4	265.9	280.1	302.1	371.3	287.9
S.D.		7.2	9.3	15.3	16.6	17.9	23.1	15.6
N=		25	25	23	23	21	21	21

*** DEAD ANIMAL.
 ANIMALS WITHOUT VIABLE FETUSES ON DAY 21 WERE EXCLUDED FROM ANALYSIS
 21(C) = DAY 21 BODY WT - GRAVID UTERUS WT

Table A- 8 (continued)

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Individual Gestation Body Weights (G) -Part II

DOSE MG/KG/DAY	ANIMAL NUMBER	DAY OF GESTATION									
		0	6	9	12	16	21	21 (C)			
833	96A0882	219.0	247.6	267.9	286.7	307.2	364.2	279.7			
	96A0883	223.0	254.7	266.7	284.9	304.4	376.8	269.8			
	96A0884	220.0	243.6	266.0	292.4	326.7	405.1	302.3			
	96A0885	229.0	259.7	273.8	296.7	328.4	396.3	301.6			
	96A0886	227.0	261.6	271.8	281.1	320.7	388.9	296.0			
	96A0887	225.0	264.7	282.0	301.4	332.1	398.1	301.6			
	96A0888	222.0	265.5	277.9	308.1	324.4	390.5	304.1			
	96A0889	219.0	248.5	267.4	280.1	313.1	383.1	293.1			
	96A0890	221.0	257.2	282.2	299.1	348.6	434.8	315.2			
	96A0891	221.0	253.8	268.2	286.8	329.1	406.9	311.4			
96A0892	216.0	255.9	268.3	290.9	328.4	406.8	303.7				
96A0893	233.0	279.2	292.4	330.5	390.9	409.4	305.1				
96A0894	210.0	235.2	250.6	261.6	290.9	353.5	270.1				
96A0895	218.0	262.8	260.6	278.3	307.8	392.5	296.9				
96A0896	241.0	244.5	280.0	291.1	325.3	394.0	297.4				
96A0897	208.0	245.8	260.1	277.2	302.6	367.9	271.0				
96A0898	227.0	254.8	267.6	273.9	290.7	341.9	274.6				
96A0899	219.0	249.1	267.2	285.0	313.4	379.7	297.4				
96A0900	220.0	243.7	261.6	285.0	311.9	371.1	287.6				
96A0901	239.0	273.4	291.2	319.5	353.0	438.7	329.9				
96A0902	215.0	248.3	264.9	285.2	311.8	379.7	293.4				
96A0903	221.0	251.0	266.0	290.9	316.9	383.4	286.9				
96A0904	214.0	243.7	259.3	278.5	304.1	375.8	288.6				
96A0905	211.0	250.2	260.7	281.4	310.8	378.5	296.7				
96A0906	226.0	246.2	261.0	281.1	304.9	373.8	291.6				
MEAN	221.8	252.9	268.9	287.5	317.5	387.9	294.6				
S.D.	8.1	8.9	9.1	11.7	15.4	22.1	14.4				
N=	25	25	25	25	25	25	25				

ANIMALS WITHOUT VIABLE FETUSES ON DAY 21 WERE EXCLUDED FROM ANALYSIS

21 (C) = DAY 21 BODY WT - GRAVID UTERUS WT

Table A- 8 (continued)

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Individual Gestation Body Weights (G) -Part II
 DAY OF GESTATION

DOSE MG/KG/DAY	ANIMAL NUMBER	0	6	9	12	16	21	21(C)
2500	96A0907	221.0	257.8	278.7	300.0	320.5	390.9	297.9
	96A0908	231.0	251.0	271.5	281.7	308.5	383.4	281.4
	96A0909	218.0	243.0	263.1	276.9	303.7	360.5	291.8
	96A0910	222.0	254.1	260.6	276.4	305.3	358.7	294.9
	96A0911	223.0	261.2	277.8	285.2	318.5	389.0	301.4
	96A0912	218.0	252.2	259.8	274.3	279.6	325.7	298.9
	96A0913	229.0	257.7	268.4	283.7	303.2	356.7	293.9
	96A0914	225.0	255.1	260.7	267.1	292.7	347.7	293.5
	96A0915	232.0	264.7	268.9	274.0	305.9	363.0	297.0
	96A0916	217.0	237.1	251.1	255.4	277.9	332.2	275.5
	96A0917	221.0	241.9	254.1	261.9	287.2	342.7	276.6
	96A0918	220.0	249.1	265.9	275.1	308.9	379.1	295.0
	96A0919	206.0	247.1	257.8	268.8	292.8	337.6	284.5
	96A0920	209.0	236.6	247.3	258.7	289.0	337.9	276.9
	96A0921	239.0	274.5	289.6	305.5	336.0	410.4	317.8
	96A0922	213.0	246.9	252.5	261.5	280.4	324.9	265.8
	96A0923	223.0	248.5	249.4	262.8	258.9	253.6	---
	96A0924	218.0	258.6	267.1	284.1	305.3	368.5	293.5
	96A0925	228.0	265.4	281.5	303.3	321.8	394.6	314.6
	96A0926	219.0	240.5	249.5	264.6	283.5	344.8	288.1
	96A0927	219.0	259.9	277.9	297.5	329.7	400.6	310.3
	96A0928	214.0	239.0	247.9	263.7	284.7	344.7	272.7
	96A0929	210.0	245.0	258.9	272.7	294.3	338.8	292.4
	96A0930	227.0	258.0	268.6	283.5	298.4	350.1	284.0
	96A0931	217.0	249.1	263.2	280.3	314.4	393.7	301.7
MEAN		220.7	251.9	264.3	277.3	301.8	361.5	291.7
S.D.		7.8	9.8	11.3	14.0	16.2	25.5	13.0
N=		24	24	24	24	24	24	24

--- NO DATA.
 0 VALUES EXCLUDED FROM ANALYSIS.
 ANIMALS WITHOUT VIABLE FETUSES ON DAY 21 WERE EXCLUDED FROM ANALYSIS
 21(C) = DAY 21 BODY WT - GRAVID UTERUS WT

Table A - 9

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Individual Gestation Body Weight Gains (G) -Part II

DOSE MG/KG/DAY	ANIMAL NUMBER	DAY OF GESTATION									
		0-6	6-9	9-12	12-16	16-21	6-16	0-21			
0	96A0832	22.7	27.6	17.4	29.7	76.1	74.7	173.5			
	96A0833	31.1	21.6	22.2	23.4	67.5	67.2	165.8			
	96A0834	28.6	17.1	18.1	22.3	72.7	57.5	158.8			
	96A0835	42.8	19.7	21.1	26.5	66.4	67.3	176.5			
	96A0836	38.3	19.2	13.9	38.9	72.5	72.0	182.8			
	96A0837	34.1 ⁰	11.7 ⁰	4.9 ⁰	1.2 ⁰	2.1 ⁰	17.8 ⁰	54.0 ⁰			
	96A0838	51.9	19.8	21.4	34.5	79.2	75.7	206.8			
	96A0839	53.5	14.5	12.0	34.7	77.9	61.2	192.6			
	96A0840	19.4	10.7	19.6	31.2	50.1 [#]	61.3	131.0			
	96A0841	25.7	19.1	14.0	39.7	75.3	72.8	173.8			
	96A0842	38.2	21.9	17.3	35.0	80.5	74.2	192.9			
	96A0843	29.5	17.7	25.4	40.3	69.7	83.4	182.6			
	96A0844	31.2	15.3	19.5	24.5	72.3	59.3	162.8			
	96A0845	27.6	13.7	12.3	29.1	74.5	55.1	157.2			
	96A0846	34.8	16.6	12.3	35.7	82.0	64.6	181.4			
	96A0847	24.2	12.3	10.6	29.4	73.8	52.3	150.3			
	96A0848	25.7	11.4	18.6	27.5	77.9	57.5	161.1			
	96A0849	30.2	17.8	18.3	38.5	84.4	74.6	189.2			
	96A0850	31.4	18.1	23.0	29.4	78.1	70.5	180.0			
	96A0851	37.6	19.7	20.8	26.0	59.5	66.5	163.6			
	96A0852	9.7	33.9	20.5	32.1	86.0	86.5	182.2			
	96A0853	31.1	20.3	34.1	31.1	80.1	85.5	196.7			
	96A0854	41.7	27.3	32.2	34.0	80.7	88.3	210.7			
	96A0855	26.7	19.4	32.2	27.3	73.2	78.9	178.8			
	96A0856	22.9	8.2	13.6	23.7	66.8	45.5	135.2			
	MEAN	31.5	18.5	19.4	31.0	74.1	68.9	174.4			
	S.D.	9.8	5.7	6.1	5.4	8.0	11.3	19.9			
	N=	24	24	24	24	24	24	24			

0 VALUES EXCLUDED FROM ANALYSIS.
 # STATISTICAL OUTLIERS INCLUDED IN ANALYSIS.
 ANIMALS WITHOUT VIABLE FETUSES ON DAY 21 WERE EXCLUDED FROM ANALYSIS

Table A- 9 (continued)

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Individual Gestation Body Weight Gains (G) -Part II

DOSE MG/KG/DAY	ANIMAL NUMBER	DAY OF GESTATION									
		0-6	6-9	9-12	12-16	16-21	6-16	0-21			
650	96A0857	29.2	30.4	20.8	***	***	***	***	62.5	159.8	
	96A0858	28.1	22.8	16.0	23.7	69.2	64.6	168.2			
	96A0859	37.7	21.7	23.9	19.0	65.9	64.6	168.2			
	96A0860	31.1	19.4	15.9	-3.6	43.6	31.7	106.4			
	96A0861	23.5	16.1	5.2	-2.5	83.2	18.8	125.5			
	96A0862	37.3	***	***	***	***	***	***			
	96A0863	35.7	11.1	12.0	31.0	57.1	54.1	146.9			
	96A0864	34.9	26.1	21.4	-0.3	88.8	47.2	170.9			
	96A0865	27.4	13.3	12.0	4.2	79.5	29.5	136.4			
	96A0866	28.6	2.4	15.7	41.5	79.7	59.6	167.9			
	96A0867	37.4	22.5	10.5	27.3	61.0	60.3	158.7			
	96A0868	40.0	16.8	10.9	35.0	63.6	62.7	166.3			
	96A0869	30.9	15.2	10.7	19.4	59.1	45.3	135.3			
	96A0870	18.0	14.7	11.6	19.5	59.8	45.8	123.6			
	96A0871	38.2	20.2	17.5	32.9	83.2	70.6	192.0			
	96A0872	31.9	9.0	16.6	28.3	67.3	53.9	153.1			
	96A0873	30.2	10.6	21.2	15.0	80.5	46.8	157.5			
	96A0874	20.8	***	***	***	***	***	***			
	96A0875	27.5	13.5	13.5	20.5	44.2	47.5	119.2			
	96A0876	26.5	20.6	-20.5#	55.3	79.7	55.4	161.6			
96A0877	31.2	10.7	16.3	21.6	61.4	48.6	141.2				
96A0878	21.8	12.4	7.1	***	***	***	***				
96A0879	34.8	6.9	21.5	26.7	81.6	55.1	171.5				
96A0880	35.0	7.8	16.1	33.6	71.5	57.5	164.0				
96A0881	27.0	-15.1#	29.6	16.6	72.8	31.1	130.9				
MEAN		30.6	14.3	14.2	22.1	69.2	49.9	150.3			
S.D.		5.8	9.2	9.4	14.6	12.6	13.1	21.4			
N=		25	23	23	21	21	21	21			

*** DEAD ANIMAL.
 # STATISTICAL OUTLIERS INCLUDED IN ANALYSIS.
 ANIMALS WITHOUT VIABLE FETUSES ON DAY 21 WERE EXCLUDED FROM ANALYSIS

Table A - 9 (continued)

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Individual Gestation Body Weight Gains (G) -Part II

DOSE MG/KG/DAY	ANIMAL NUMBER	DAY OF GESTATION									
		0-6	6-9	9-12	12-16	16-21	6-16	0-21			
833	96A0882	28.6	20.3	18.8	20.5	57.0	59.6	145.2			
	96A0883	31.7	12.0	18.2	19.5	72.4	49.7	153.8			
	96A0884	23.6	22.4	26.4	34.3	78.4	83.1	185.1			
	96A0885	30.7	14.1	22.9	31.7	67.9	68.7	167.3			
	96A0886	34.6	10.2	9.3	39.6	68.2	59.1	161.9			
	96A0887	39.7	17.3	19.4	30.7	66.0	67.4	173.1			
	96A0888	43.5	12.4	30.2	16.3	66.1	58.9	168.5			
	96A0889	29.5	18.9	12.7	33.0	70.0	64.6	164.1			
	96A0890	36.2	25.0	16.9	49.5	86.2	91.4	213.8			
	96A0891	32.8	14.4	18.6	42.3	77.8	72.5	185.9			
	96A0892	39.9	12.4	22.6	37.5	78.4	72.5	190.8			
	96A0893	28.8	17.4	13.2	38.1	78.9	68.7	176.4			
	96A0894	25.2	15.4	11.0	29.3	62.6	55.7	143.5			
	96A0895	44.8	-2.2* 3.5#	17.7	29.5	84.7	45.0	174.5			
	96A0896	3.5#	35.5#	11.1	34.2	68.7	80.8	153.0			
	96A0897	37.8	14.3	17.1	25.4	65.3	56.8	159.9			
	96A0898	27.8	12.8	6.3	25.4	51.2	35.9	114.9			
	96A0899	30.1	18.1	16.8	29.4	73.5	64.3	167.9			
	96A0900	23.7	17.9	23.4	26.9	59.2	68.2	151.1			
	96A0901	34.4	17.8	28.3	33.5	85.7	79.6	199.7			
96A0902	33.3	16.6	20.3	26.6	67.9	63.5	164.7				
96A0903	30.0	15.0	24.9	26.0	66.5	65.9	162.4				
96A0904	29.7	15.6	19.2	25.6	71.7	60.4	161.8				
96A0905	39.2	10.5	20.7	29.4	67.7	60.6	167.5				
96A0906	20.2	14.8	20.1	23.8	68.9	58.7	147.8				
MEAN	31.2	16.0	18.6	30.0	70.4	64.6	166.2				
S.D.	8.5	6.5	5.9	7.9	8.7	12.1	19.9				
N=	25	25	25	25	25	25	25				

* STATISTICAL OUTLIERS INCLUDED IN ANALYSIS.
 # ANIMALS WITHOUT VIABLE FETUSES ON DAY 21 WERE EXCLUDED FROM ANALYSIS

Table A- 9 (continued)

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Individual Gestation Body Weight Gains (G) -Part II

DOSE MG/KG/DAY	ANIMAL NUMBER	DAY OF GESTATION									
		0-6	6-9	9-12	12-16	16-21	6-16	0-21			
2500	96A0907	36.8	20.9	21.3	20.5	70.4	62.7	169.9			
	96A0908	20.0	20.5	10.2	26.8	74.9	57.5	152.4			
	96A0909	25.0	20.1	13.8	26.8	56.8	60.7	142.5			
	96A0910	32.1	6.5	15.8	28.9	53.4	51.2	136.7			
	96A0911	38.2	16.6	7.4	33.3	70.5	57.3	166.0			
	96A0912	34.2	7.6	14.5	5.3	46.1	27.4	107.7			
	96A0913	28.7	10.7	15.3	19.5	53.5	45.5	127.7			
	96A0914	30.1	5.6	6.4	25.6	55.0	37.6	122.7			
	96A0915	32.7	4.2	5.1	32.9	56.1	42.2	131.0			
	96A0916	20.1	14.0	4.3	22.5	54.3	40.8	115.2			
	96A0917	20.9	12.2	7.8	25.3	55.5	45.3	121.7			
	96A0918	29.1	16.8	9.2	33.8	70.2	59.8	159.1			
	96A0919	41.1	10.7	11.0	24.0	44.8	45.7	131.6			
	96A0920	27.6	10.7	11.4	30.3	48.9	52.4	128.9			
	96A0921	35.5	15.1	15.9	30.5	74.4	61.5	171.4			
	96A0922	33.9	5.6	9.0	18.9	44.5	33.5	111.9			
	96A0923	25.5 ⁰	0.9 ⁰	13.4 ⁰	-3.9 ⁰	-5.3 ⁰	10.4 ⁰	30.6 ⁰			
	96A0924	40.6	8.5	17.0	21.2	63.2	46.7	150.5			
	96A0925	37.4	16.1	21.8	18.5	72.8	56.4	166.6			
	96A0926	21.5	9.0	15.1	18.9	61.3	43.0	125.8			
96A0927	40.9	18.0	19.6	32.2	70.9	69.8	181.6				
96A0928	25.0	8.9	15.8	21.0	60.0	45.7	130.7				
96A0929	35.0	13.8	13.9	21.6	44.5	49.3	128.8				
96A0930	31.0	10.6	14.9	14.9	51.7	40.4	123.1				
96A0931	32.1	14.1	17.1	34.1	79.3	65.3	176.7				
MEAN		31.2	12.4	13.1	24.5	59.7	49.9	140.8			
S.D.		6.6	4.9	4.9	7.0	10.8	10.6	22.0			
N=		24	24	24	24	24	24	24			

0 VALUES EXCLUDED FROM ANALYSIS.
 0 ANIMALS WITHOUT VIABLE FETUSES ON DAY 21 WERE EXCLUDED FROM ANALYSIS

Table A-10

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYOXAL ANION AND METABOLIC ACIDOSIS

Individual Organ And Organ/Body Weights-Part II

DOSE MG/KG/DAY	ANIMAL NUMBER	FINAL		KIDNEYS		LIVER	
		BODY WT. (G)	(G)	(G/100)	(G)	(G/100)	
0	96A0832	407	1.902	0.468	13.354	3.285	
	96A0833	390	1.733	0.445	14.910	3.825	
	96A0834	380	1.643	0.433	12.307	3.240	
	96A0835	392	1.857	0.474	13.813	3.528	
	96A0836	414	1.940	0.469	14.023	3.389	
	96A0837	280 ^ø	2.194 ^ø	0.784 ^ø	13.418 ^ø	4.792 ^ø	
	96A0838	424	1.701	0.401	14.411	3.400	
	96A0839	412	1.871	0.455	13.653	3.317	
	96A0840	336	1.521	0.453	10.116	3.011	
	96A0841	398	1.891	0.475	13.078	3.288	
	96A0842	412	2.103	0.511	14.317	3.476	
	96A0843	410	2.181	0.532	13.456	3.285	
	96A0844	377	2.171	0.576	15.066	3.998	
	96A0845	366	2.012	0.549	13.792	3.766	
	96A0846	408	2.315	0.575	14.835	3.632	
	96A0847	360	2.072	0.567	13.329	3.699	
	96A0848	386	1.699	0.440	13.156	3.407	
	96A0849	405	1.884	0.465	15.887	3.921	
	96A0850	411	1.947	0.474	13.925	3.388	
	96A0851	384	1.704	0.444	12.878	3.357	
	96A0852	392	1.765	0.450	13.543	3.453	
	96A0853	421	2.265	0.538	14.362	3.414	
	96A0854	424	2.201	0.519	16.002	3.777	
	96A0855	399	1.987	0.498	13.791	3.458	
	96A0856	351	1.677	0.478	11.732	3.341	
	MEAN	394	1.918	0.487	13.739	3.486	
	S.D.	23	0.215	0.048	1.260	0.239	
	N=	24	24	24	24	24	

ø VALUES EXCLUDED FROM ANALYSIS.
 ø ANIMALS WITHOUT VIABLE FETUSES ON DAY 21 WERE EXCLUDED FROM ANALYSIS

Table A-10 (continued)

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Individual Organ And Organ/Body Weights-Part II

DOSE MG/KG/DAY	ANIMAL NUMBER	FINAL BODY WT. (G)		KIDNEYS		LIVER	
		(G)	(G)	(G/100)	(G)	(G/100)	
650	96A0858	389	1.942	0.499	16.298	4.192	
	96A0859	384	1.821	0.474	15.216	3.960	
	96A0860	326	2.043	0.626	14.800	4.534	
	96A0861	344	1.901	0.553	14.328	4.171	
	96A0863	378	2.106	0.557	12.643	3.346	
	96A0864	395	1.827	0.463	15.836	4.010	
	96A0865	370	2.044	0.552	12.614	3.406	
	96A0866	385	1.921	0.499	14.864	3.862	
	96A0867	383	2.070	0.541	16.015	4.185	
	96A0868	385	1.906	0.495	14.158	3.675	
	96A0869	345	2.022	0.586	14.058	4.071	
	96A0870	343	1.596	0.466	10.580	3.088	
	96A0871	412	2.299	0.558	16.710	4.056	
	96A0872	362	1.886	0.515	15.196	4.197	
	96A0873	384	2.038	0.531	15.434	4.025	
	96A0875	332	1.726	0.520	12.902	3.884	
	96A0876	395	1.804	0.457	15.763	3.995	
	96A0877	369	2.128	0.576	15.589	4.222	
	96A0879	389	2.121	0.546	16.347	4.208	
	96A0880	378	1.989	0.526	15.823	4.186	
	96A0881	350	1.943	0.555	12.994	3.714	
	MEAN	371	1.958	0.528	14.675	3.952	
	S.D.	23	0.157	0.044	1.567	0.343	
	N=	21	21	21	21	21	

ANIMALS WITHOUT VIABLE FETUSES ON DAY 21 WERE EXCLUDED FROM ANALYSIS

Table A-10 (continued)

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

Individual Organ And Organ/Body Weights-Part II

DOSE MG/KG/DAY	ANIMAL NUMBER	FINAL		KIDNEYS		LIVER	
		BODY WT. (G)	(G)	(G/100)	(G)	(G/100)	
833	96A0882	364	1.862	0.511	13.179	3.619	
	96A0883	377	1.922	0.510	13.575	3.603	
	96A0884	405	2.026	0.500	13.458	3.322	
	96A0885	396	2.056	0.519	15.684	3.958	
	96A0886	389	1.997	0.513	16.657	4.283	
	96A0887	398	2.108	0.530	15.355	3.857	
	96A0888	391	1.840	0.471	15.804	4.047	
	96A0889	383	1.885	0.492	14.020	3.660	
	96A0890	435	2.025	0.466	15.966	3.672	
	96A0891	407	2.068	0.508	15.829	3.890	
	96A0892	409	1.908	0.469	16.336	4.016	
	96A0893	409	1.851	0.452	13.957	3.409	
	96A0894	354	1.929	0.546	13.677	3.869	
	96A0895	393	1.878	0.478	14.337	3.653	
	96A0896	394	2.084	0.529	17.277	4.385	
	96A0897	368	1.828	0.497	15.135	4.114	
	96A0898	342	1.772	0.518	13.441	3.931	
	96A0899	387	1.877	0.485	16.910	4.371	
	96A0900	371	1.697	0.457	12.154	3.275	
	96A0901	439	2.239	0.510	18.026	4.109	
	96A0902	380	2.073	0.546	13.615	3.586	
	96A0903	383	2.052	0.535	16.984	4.430	
	96A0904	376	2.093	0.557	14.204	3.780	
	96A0905	379	2.276	0.601	16.570	4.378	
	96A0906	374	1.910	0.511	13.891	3.716	
	MEAN	388	1.970	0.509	15.042	3.877	
	S.D.	22	0.139	0.034	1.555	0.334	
	N=	25	25	25	25	25	

ANIMALS WITHOUT VIABLE FETUSES ON DAY 21 WERE EXCLUDED FROM ANALYSIS

Table A-10 (continued)

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
ON THE ROLE OF GLYOXALATE ANION AND METABOLIC ACIDOSIS

THE DOW CHEMICAL COMPANY
STUDY ID: K-002558-012
PAGE: 105

Individual Organ And Organ/Body Weights-Part II

DOSE MG/KG/DAY	ANIMAL NUMBER	BODY FINAL		KIDNEYS		LIVER	
		WT. (G)	(G)	(G/100)	(G)	(G/100)	
2500	96A0907	391	2.262	0.579	15.170	3.881	
	96A0908	383	2.106	0.549	14.032	3.660	
	96A0909	361	1.920	0.533	14.669	4.069	
	96A0910	359	2.091	0.583	16.006	4.462	
	96A0911	389	1.968	0.506	16.698	4.293	
	96A0912	326	2.442	0.750	17.323	5.319#	
	96A0913	357	2.358	0.661	15.865	4.448	
	96A0914	348	1.939	0.558	14.637	4.210	
	96A0915	363	1.975	0.544	15.290	4.212	
	96A0916	332	1.801	0.542	12.376	3.725	
	96A0917	343	1.656	0.483	12.488	3.644	
	96A0918	379	1.902	0.502	14.285	3.768	
	96A0919	338	1.873	0.555	14.347	4.250	
	96A0920	338	1.940	0.574	13.488	3.992	
	96A0921	410	2.254	0.549	17.151	4.179	
	96A0922	325	2.225	0.685	13.962	4.297	
	96A0923	254#	2.113#	0.833#	9.923#	3.913#	
	96A0924	369	1.932	0.524	14.990	4.068	
	96A0925	395	2.009	0.509	14.027	3.555	
	96A0926	345	2.294	0.665	15.664	4.543	
	96A0927	401	2.329	0.581	16.160	4.034	
	96A0928	345	2.005	0.582	14.755	4.281	
	96A0929	339	2.330	0.688	14.600	4.309	
	96A0930	350	1.911	0.546	13.461	3.845	
	96A0931	394	2.395	0.608	16.968	4.310	
	MEAN	362	2.080	0.577	14.934	4.140	
	S.D.	25	0.213	0.067	1.360	0.375	
	N=	24	24	24	24	24	

g VALUES EXCLUDED FROM ANALYSIS.
STATISTICAL OUTLIERS INCLUDED IN ANALYSIS.
ANIMALS WITHOUT VIABLE FETUSES ON DAY 21 WERE EXCLUDED FROM ANALYSIS

Table A-11

ETHYLENE GLYCOL DEVELOPMENTAL TOXICITY: MECHANISTIC STUDY
 ON THE ROLE OF GLYCOLATE ANION AND METABOLIC ACIDOSIS

INDIVIDUAL ANIMAL DATA

ANIMAL: 96A0832 DOSE: 0 MG/KG/DAY C-SECTION-DATE: 02/27/96

MATERIAL OBSERVATIONS: KIDNEY CYST CORTEX RIGHT PREGNANCY STATUS: PREGNANT, ALL NORMAL

ORGAN WEIGHT (g): UTERUS(GRAVID): 112.30

LIVER: 13.35

KIDNEYS: 1.90

CORPORA LUTEA: RIGHT 8 LEFT 10 TOTAL 18

IMPLANTATIONS: 8 8 16

IMPLANT #	STATUS	SEX	WEIGHT(G)	OBSERVATIONS/COMMENTS
01	ALIVE	FEMALE	4.96	EXTERNAL: NVL SKELETAL: NVL
02	ALIVE	FEMALE	5.19	EXTERNAL: NVL SKELETAL: CENTRA-D.O.:CERVICAL, # 4 STERNEBRAE-D.O.: # 5
03	ALIVE	FEMALE	5.60	EXTERNAL: NVL SKELETAL: NVL
04	ALIVE	FEMALE	5.57	EXTERNAL: NVL VISCERAL: NVL BOJINS: NVL
05	ALIVE	MALE	5.60	EXTERNAL: NVL VISCERAL: NVL BOJINS: NVL
06	ALIVE	FEMALE	4.83	EXTERNAL: NVL SKELETAL: NVL
07	ALIVE	FEMALE	5.43	EXTERNAL: NVL SKELETAL: NVL
08	ALIVE	MALE	5.73	EXTERNAL: NVL VISCERAL: NVL BOJINS: NVL
09	ALIVE	FEMALE	5.65	EXTERNAL: NVL SKELETAL: SKULL-D.O.: OCCIPITAL RIBS-D.O.: # 11, RIGHT
10	ALIVE	MALE	5.23	EXTERNAL: NVL VISCERAL: NVL BOJINS: NVL
11	ALIVE	MALE	5.36	EXTERNAL: NVL SKELETAL: NVL

CECATS/TRIAGE TRACKING DATABASE ENTRY FORM

CECATS DATA:

Submission # 8EHO-F41-0497-032.3 SEQ K

TYPE: INT SUPP (FLWP)

SUBMITTER NAME: _____

Union Carbide Corporation

SUB DATE: 3-27-97 OTS DATE: 4-1-97

CHEMICAL NAME 3 words

Ethylene Glycol

glycolic acid

Sodium glycolate

INFORMATION REQUESTED FLWP DATE

- 0501 NO INFO REQUESTED
- 0502 INFO REQUESTED (TECH)
- 0503 INFO REQUESTED (VOL ACTIONS)
- 0504 INFO REQUESTED (REPORTING RATIONALE)

DISPOSITION

- 0639 REFER TO CHEMICAL SCREENING
- 0678 CAP NOTICE

CSRAD DATE: 8-13-97

VOLUNTARY ACTIONS

- 0401 NO ACTION REPORTED
- 0402 STUDIES PLANNED/UNDERWAY
- 0403 NOTIFICATION OF WORKER/OTHERS
- 0404 LABEL/MSDS CHANGES
- 0406 PROCESS/HANDLING CHANGES
- 0407 PRODUCTION DISCONTINUED
- 0408 CONFIDENTIAL

CAS #

107-21-1

79-14-1

2836-32-0

INFORMATION TYPE:

- 0201 ONCO (HUMAN)
- 0202 ONCO (ANIMAL)
- 0203 CELL TRANS (IN VITRO)
- 0204 MUTA (IN VITRO)
- 0205 MUTA (IN VIVO)
- 0206 REPRO/TERATO (HUMAN)
- 0207 REPRO/TERATO (ANIMAL)
- 0208 NEURO (HUMAN)
- 0209 NEURO (ANIMAL)
- 0210 ACUTE TOX. (HUMAN)
- 0211 CHR. TOX. (HUMAN)
- 0212 ACUTE TOX. (ANIMAL)
- 0213 SUBACUTE TOX. (ANIMAL)
- 0214 SUB CHRONIC TOX. (ANIMAL)
- 0215 CHRONIC TOX. (ANIMAL)

(P F C)

- 01 02 04
- 01 02 04
- 01 02 04
- 01 02 04
- 01 02 04
- 01 02 04
- 01 02 04
- 01 02 04
- 01 02 04
- 01 02 04
- 01 02 04
- 01 02 04
- 01 02 04
- 01 02 04
- 01 02 04

INFORMATION TYPE:

- 0216 EPI/CLIN
- 0217 HUMAN EXPOS (PROD CONTAM)
- 0218 HUMAN EXPOS (ACCIDENTAL)
- 0219 HUMAN EXPOS (MONITORING)
- 0220 ECO/AQUA TOX.
- 0221 ENV. OCC/REL/FATE
- 0222 EMER. INCI. OF ENV. CONTAM.
- 0223 RESPONSE REQUEST DELAY
- 0224 PROD/COMP/CHEM ID
- 0225 REPORTING RATIONALE
- 0226 CONFIDENTIAL
- 0227 ALLERG (HUMAN)
- 0228 ALLERG (ANIMAL)
- 0239 METAB/PHARMACO (ANIMAL)
- 0240 METAB/PHARMACO (ANIMAL)

P F C

- 01 02 04
- 01 02 04
- 01 02 04
- 01 02 04
- 01 02 04
- 01 02 04
- 01 02 04
- 01 02 04
- 01 02 04
- 01 02 04
- 01 02 04
- 01 02 04
- 01 02 04
- 01 02 04
- 01 02 04
- 01 02 04

INFORMATION TYPE:

- 0241 IMMUNO (ANIMAL)
- 0242 IMMUNO (HUMAN)
- 0243 CHEM/PHYS PROP
- 0244 CLASTO (IN VITRO)
- 0245 CLASTO (ANIMAL)
- 0246 CLASTO (HUMAN)
- 0247 DNA DAM/REPAIR
- 0248 PROD/USE/PROC
- 0251 MSDS
- 0299 OTHER

P F C

- 01 02 04
- 01 02 04
- 01 02 04
- 01 02 04
- 01 02 04
- 01 02 04
- 01 02 04
- 01 02 04
- 01 02 04
- 01 02 04

TRIAGE DATA: NON-CBI INVENTORY:

- YES (CONTINUE)
- NO (DROP)
- DETERMINE

ONGOING REVIEW:

- YES (DROP/REFER)
- NO (CONTINUE)
- REFER

SPECIES:

Rat

TOXICOLOGICAL CONCERN:

- LOW
- MEDIUM
- HIGH

USE:

PRODUCTION:

COMMENTS:

TRIAGE of 8(e) Submissions

Date sent to triage: _____

NON-CAP

CAP

Submission number: FYI - 0323 K

TSCA Inventory (Y) N D

STUDY TYPE (circle appropriate):

Cheng-Chun Lee (E609C)

ATOX SBTOX SEN W/NEUR

Larry Newsome (E425)

ECO AQUATO

Katherine Anitole (E611G)

RTOX/DTOX

Daljit Sawhney (E611A)

CTOX STOX

Deborah Norris (E602)

NEUR

Jeff Beaubier (E608)

EPI

Ron Ward (E611F)

IMMUNO/ALLERG

Davis Lai (E611B)

CARC

Michael Cimino (E611D)

GTOX

Leonard Keifer (E611C)

META/PHARM

NOTES: 3 records